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TEXAS MEMORIAL MUSEUM

**INTERACTIONS BETWEEN A BISEXUAL FISH SPECIES
AND ITS GYNOGENETIC SEXUAL PARASITE**



by

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and Its Gynogenetic Sexual Parasite

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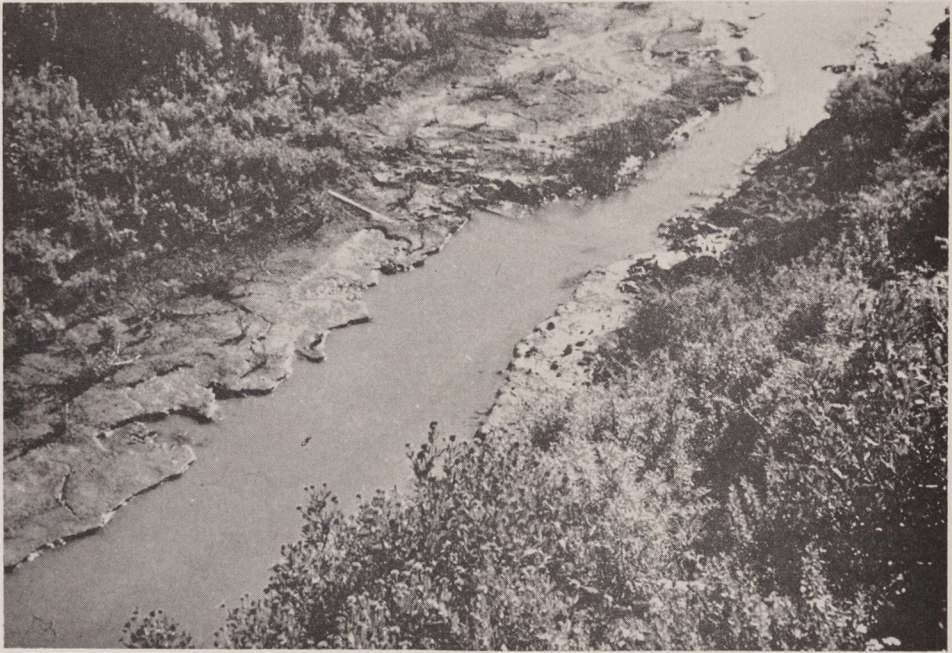
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A. Typical habitat of *Mollienesia* in the Brownsville area. Photograph of a part of Segment A of Station 2. Note the muddy water and shoreline. The stream is two to four feet wide. Photo by Richard Hewitt.



B. Photograph of Segment B of Station 2 from the junction. Vegetation is typical of ditches near Brownsville; water by the right bank is two to three inches deep. Photo by Mrs. H. G. Merriam.

Interactions Between a Bisexual Fish Species and Its Gynogenetic Sexual Parasite

Introduction

Gynogenesis, embryonic development without functional paternal chromatin, occurs in a large number of animal phyla but has not been shown to be widely established as the normal method of reproduction in any major group of animals (White, 1954). Gynogenetic populations are exclusively female or mostly females with rare males. All (or almost all) offspring develop from ova that could not have been fertilized by homospecific males. Many gynogenetic populations are able to reproduce in the absence of any males. This would include cyclical parthenogenesis of White (1954) and many of his examples of complete parthenogenesis. Two of the gynogenetic species, the beetle *Ptinus latro* (Moore, Woodroffe, and Sanderson, 1956) and the fish *Mollienesia formosa* (Hubbs and Hubbs, 1932), are sexual parasites. Virgin females of each will not produce viable offspring; however, females of both will do so after mating with males of a related bisexual species. The resulting offspring have the phenotype of the maternal species and show no indication of functional paternal chromatin. The amazon molly (*M. formosa*) has been studied for several years and through twenty-one generations without any paternal inheritance (Hubbs, 1955). Females separated from males by glass plates or other objects that prevented contact remained virgin, showing that contact (copulation), not behavior phenomena, is involved in stimulating development (Meyer, 1938).

Hubbs and Hubbs (1932) suggested that *M. formosa* is of hybrid origin and that *M. latipinna* and a member of the *M. sphenops* species group (*M. mexicana* would be the logical species) are the parental species. The amazon molly is intermediate between *M. latipinna* and the *M. sphenops* species group and hybrids between these bisexual forms include sterile males and partly fertile females. These females produce bisexual broods that show the influence of paternal chromatin but are otherwise so similar to females of *M. formosa* that they cannot be distinguished from each other. Likewise F_1 hybrid males are remarkably like females of *M. formosa* that have been masculinized by male hormones and are also like the rare wild-caught "males" of *M. formosa*. Furthermore, Hewitt *et al.* (1963) have shown that the electrophoretic patterns of the muscle of *M. formosa* is in many ways intermediate between that of *M. latipinna* and some populations of the *M. sphenops* group. i.e., their Fig. 5 (*M. formosa*) is intermediate

between their Fig. 7 (*M. latipinna*) and Fig. 4 (*M. sphenops*). I suspect that *M. formosa* is an allodiploid having a genetic composition that permits its unusual mode of reproduction. *Ptinus latro* may also result from hybridization; however, the results are different as it is a triploid.

Gynogenetic development of the amazon molly requires the presence of (and presumably copulation by) males of related fishes. Although males of many related genera (*Gambusia*, *Poecilia*, *Lebistes*, *Allopoecilia*, and *Limia*) can act as "fathers," it is likely that natural populations are maintained chiefly by use of *Mollienesia mexicana* or *M. latipinna* males. Either one or both of these species is sympatric with *M. formosa* throughout its range—southern Texas and northeastern Mexico. Males of the bisexual species would therefore have two distinct types of females to court. As *Mollienesia* females in bisexual populations typically outnumber males, it is possible that male frequency may limit reproduction. This would obviously be most probable in the presence of the all-female sexual parasite. Both bisexual species also occur in areas remote from those they occupy together with the amazon molly. It is possible, therefore, to obtain information on the effect of a sexual parasite on a bisexual species by comparison of allopatric and sympatric populations. My study areas have been in southern Texas and have included only the sailfin molly, *M. latipinna*, and the amazon molly, *M. formosa*.

Haskins, Haskins, and Hewitt (1960) have shown that *M. formosa* will also rarely produce offspring that show the influences of paternal chromatin. One cross (of several) using *Limia vittata* males produced typical hybrids. Another cross used a *M. latipinna* male that had received a dosage of 1050 roentgens. More recent experiments have produced another example of inheritance from irradiated males. Kallman (personal communication) has also reported crosses involving *M. formosa* that show indication of paternal chromatin. One cross involved *L. vittata* males and the other paternal parents were aquarium "black mollies." This strain is a descendant of a series of hybrid matings involving a number of species of *Mollienesia*, but not *M. latipinna* or *M. mexicana*. Both Kallman and Haskins have numerous additional laboratory-reared *M. formosa* that show no sign of paternal inheritance. Therefore, only a few of the numerous laboratory broods of *M. formosa* show paternal influence. The ones involving *M. latipinna* obviously concern males that had been subjected to conditions drastically different from those in the natural habitat that could have caused the suspected paternal influence. The remaining three examples of paternal influence involve males from stocks allopatric to *M. formosa*. It is possible that a "recognition factor" functions best with sympatric fishes. Hubbs (1960 and 1961) presented evidence that "gametic recognition" functioned in sympatric interspecific crosses but did not work with allopatric crosses.

Another variant may occur in salamanders of the *Ambystoma jeffersonianum* complex (Uzzell, 1962), in which the bisexual diploid and unisexual triploid strains have not been placed in different species. Triploid females that are known in several populations have produced only female offspring when the young were reared in field pens; however, whether paternal chromatin is functional in somatic inheritance is not yet documented.

A somewhat different situation occurs in the related fish genus *Poeciliopsis* (Miller and Schultz, 1959). Two all female (or nearly so) strains occur in which the presence of (and presumably copulation by) related males is required for reproduction. All offspring produced by such females are females and have the morphologic attributes of the female strain. However, both all-female strains at present have been regarded as conspecific with the related bisexual forms (Miller, 1961). Furthermore, Schultz (1961) has shown that when females are crossed with males of another species, the offspring may have marks present in the paternal and absent in the maternal species. These inheritance mechanisms must differ from those which typically occur in *M. formosa*.

Meyer (1938) studied the histology and cytology of *M. formosa*. He showed that differential mortality could not account for the absence of male offspring. He also measured nuclear diameters and obtained approximately equal sizes for *M. formosa*, *M. sphenops* (= *M. mexicana*), and *M. latipinna*. He therefore assumed that *M. formosa* is a diploid. This assumption has been supported by Haskins, Haskins, and Hewitt (1960) and Kallman (1962a), who reported that *M. formosa* had 44–46 chromosomes (diploid), which compares favorably with Wickbom's (1943) counts of 44–46 for *M. sphenops* and *M. velifera*. The *M. formosa* counts were based on studies by R. J. Schultz and G. E. Drewry. As the details of these examinations have not been reported further, Drewry's analyses are included in the appendix of this paper. Meyer (1938) assumed that gynogenetic diploids would be exceedingly improbable because fusion of reduced female nuclei is unlikely. He therefore assumed that the paternal chromatin is present but inactive. He neglected to consider the possibility that unreduced ova could produce the next generation. Although no additional evidence is available, I consider Meyer's hypothesis unlikely. His hypothesis would assume that all paternal chromosomes are removed in the polar bodies and that crossing-over would not occur. For support, he cited several hybrid studies in which paternal chromatin has been shown to be present but early cleavage was at the maternal rate (Moenkhaus, 1904; Newman, 1915; and Pinney, 1918; etc.). Unfortunately, all such hybrids that retain the paternal chromatin through gastrulation include paternal phenotypic morphologic and physiologic attributes, and those that are reared to mature size are intermediate (Hubbs and Drewry, 1960, etc.). The examples that best support Meyer's hypothesis

die at gastrulation. This question can best be resolved by careful cytological studies using "fathers" with morphologically or chemically distinguishable chromosomes.

Males of the amazon molly have been noted in nature (Hubbs, Drewry, and Warburton, 1959). The role of the males is uncertain; however, available evidence indicates that they are genetic females which have been masculinized by environmental factors. Males can receive spleen transplants from one (and only one) of the clones occurring in the same locality (Kallman, 1962b). Evidence is presented in this paper that indicates male frequency varies with the season. Preliminary laboratory studies support the hypothesis and it is possible that cold temperature during early development has a masculinizing influence.

Kallman (1962a) has shown that tissues can be exchanged between amazon molly mothers and daughters whereas the "fathers" cannot exchange tissues with the offspring. Therefore, the functional chromatin of mothers and daughters is very similar. He has also shown (Kallman, 1962b) that there are groups of individuals in natural populations among which tissues can be exchanged. He considers each of these groups of individuals to be a clone. Several clones occur in a natural population indicating some genetic recombination or dominant mutations. If the species were a functional haploid or the mutations dominant, the mutation rate would have to be extremely slow to produce the few known clones. If it were a functional diploid, the chance occurrence of two recessive mutations at the same locus could account for the few clones. Genetic recombination is a less likely source of clone production. If reduction division does occur it could result in different haploid nuclei that later fuse. Any genetic recombination from this source would have occurred long in the past and natural selection or chance should have eliminated most genotypes. Genetic recombination after homospecific fertilization is a possibility; however, most males that have been tested have not fathered broods when presented with virgin females, and the single "functional male" may have merely stimulated gynogenetic development.

This study chiefly consists of analyses of preserved material collected at monthly intervals near Brownsville, Texas. The study was designed to contrast the reproductive efficiency of amazon and sailfin mollies collected at the same time and place. Samples of sailfins from outside the range of the amazon molly were obtained and analyzed to obtain an estimate of the effect of the gynogenetic sexual parasite on its host. Approximately 30,000 mollies were collected, providing about 500,000 eggs which were used in the relative fertility studies. More than 1,000 live mollies were brought to the laboratory to test hypotheses derived from observations on preserved fish. These studies show that an amazon molly female has a reproductive potential approximating that of a sailfin; however, the sailfins more nearly reach their potential than do the amazons.

Additional information on growth rates, breeding season, and intrapopulation variations in reproductive potential are also presented.

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The manuscript has been read and criticized by Caryl P. Haskins, Klaus D. Kallman, Robert R. Miller, and R. Jack Schultz. I have also profited by discussions of this study with W. F. Blair, George E. Drewry, Richard Hewitt, C. L. Hubbs, B. Maguire, H. G. Merriam, and numerous others. Obviously I assume full responsibility for all conclusions.

Materials and Methods

The specimens were collected from four localities at approximately monthly intervals between February, 1960, and September, 1961 (Table 1). At each locality the same approximate area was seined in the same manner with a 10-foot common sense minnow seine. All collected fishes were preserved in 10 per cent formalin. The occasional living samples were obtained from adjacent areas. The mollies in the preserved samples were sexed by determining the amount of anal fin modification and each was measured. The females were dissected to determine ovarian development. If they had eggs with yolk they were tabulated as ovulated. If the eggs had noticeable embryos, the female was considered pregnant. Many females had some mature eggs with embryos and others without. These were considered to be partially pregnant. Males were also dissected to confirm the sexual differen-

TABLE I
Collection Dates

	Station 1	Station 2	Station 3	Station 4
February, 1960	20	20	21	
March, 1960	26	26	27	
April, 1960	23	23	24	
May, 1960	21	21	22	
June, 1960	4	4	5	
July, 1960	23	23	24	24
August, 1960	13	13	14	14
September, 1960	17	17	18	18
October, 1960	9	9	10	10
November, 1960	6	6	7	7
December, 1960	4	4	5	5
January, 1961	8	8	9	
February, 1961	5	5		
March, 1961	12	12	11	13
April, 1961	16	16	16	17
May, 1961	7	7	8	8
June, 1961	4	4	5	5
June, 1961	25	25	24	24
July, 1961	23	23	22	22
August, 1961	26	26	25	25
September, 1961	24		23	23

tiation. The fish were then catalogued in the Texas Natural History Collection and the eggs discarded.

A ditch on the Lula B. Sams State Fish Hatchery at Olmito, Cameron County, Texas, was Station 1 (Fig. 1). Samples were taken from Segment A and the west end of Segment B from February to June, 1960. Segment A was covered in three seine hauls, each of which was done three times each month. The hauls were made toward the west. Segment B was covered in one seine haul toward the east which was repeated. The entire ditch was dredged between June and July, 1960, and the subsequent collections contained few fish. The July, 1960, collection was obtained from Segment A east to the pool. The entire area was seined once toward the east. In August, 1960, the fish were obtained from Segment B. Except for the July and September, 1961, collections, the remaining fish were also obtained from Segment B. The area was seined east to west and then west to east. Two hauls were made in each direction unless heavy vegetation necessitated shorter hauls. The July, 1961, collection was obtained from Segment C. Two short seine hauls contained the entire preserved sample. The September, 1961, collection was obtained from Segments B, C, and Resaca del Rancho Viejo. A 9.68" rainfall 10 days previously, a by-product of Hurricane Carla, resulted in high water

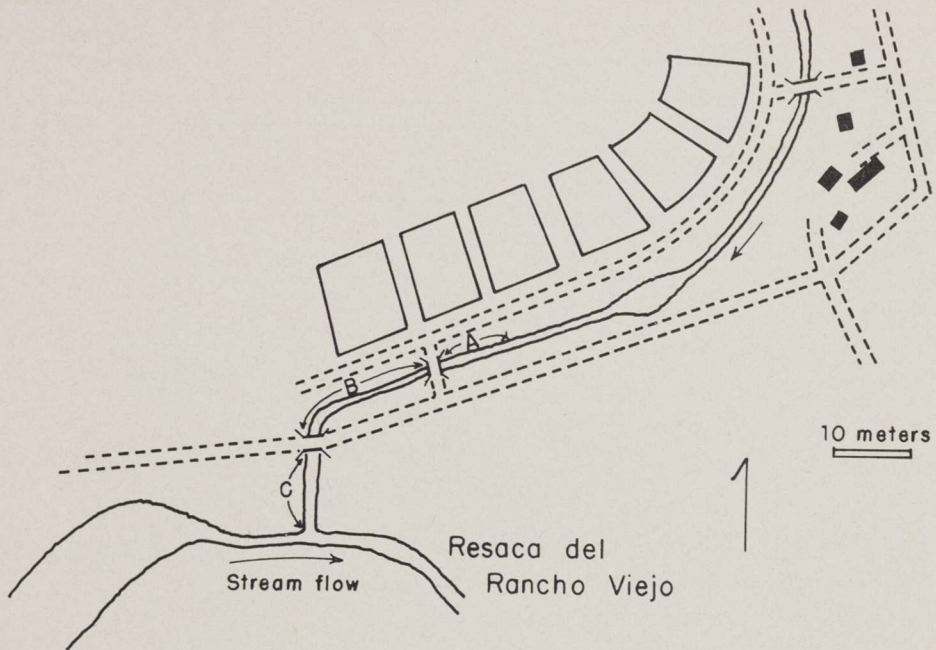


FIG. 1. Sketch map of collection station No. 1 at the Lula Sams State Fish Hatchery, Olmito, Texas.

and few fish were obtained despite more than three hours of work. The July, 1961, collection is not included in the sex ratio data, otherwise it can be recognized by the collection date. The live samples were all obtained from Segment C.

The Lula Sams State Fish Hatchery is the source of most of the stocks reported by Hubbs and Hubbs (1932) and Hubbs (1955). Likewise, Kallman (1962b) used mollies from Segment A for his studies on clonal diversity.

A ditch that crosses Central Avenue, Brownsville, Texas, about one-half mile east of the international airport was Station 2 (Fig. 2). All collections were obtained from Segments A and B. Usually Segment A had more fish in the summer and Segment B had more fish in the winter. Segment A was seined north and then south to north in four seine hauls each and Segment B was seined east to west in six seine hauls. Shorter hauls were occasionally made when vegetation was dense. This station was not sampled in September, 1961, due to the high water. The scouring action of the floods widened and deepened the ditches, Segment A changed from about four feet wide to over 15 feet wide and Segment B changed from 10 to 15 feet in width. The plank bridge marking the upper end of Segment A was removed and the pipe at the west end of Segment B was partly buried. This is the locality from which Hubbs, Drewry, and Warburton (1959) reported a male of

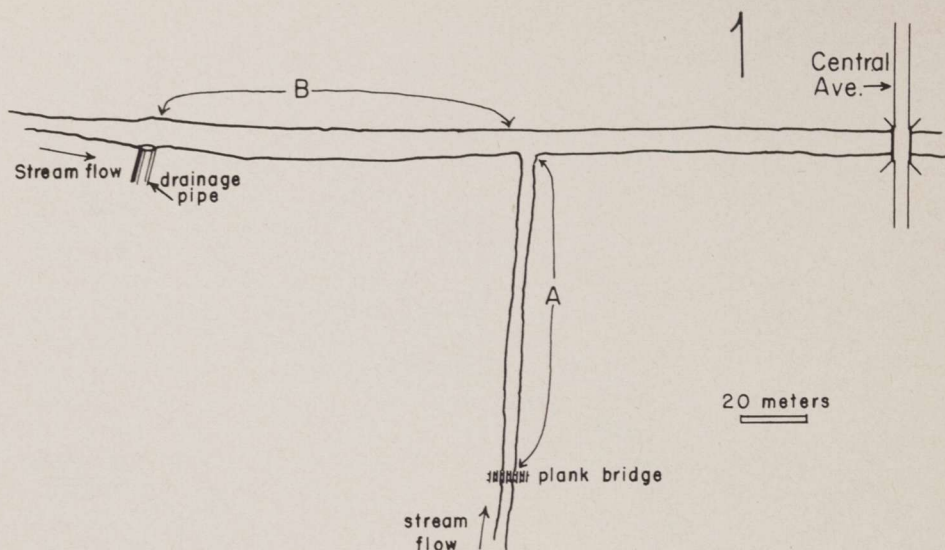


FIG. 2. Sketch map of collection station No. 2 at a drainage ditch crossing Central Avenue one-half mile west of the Brownsville International Airport.

M. formosa. The live samples were obtained just upstream from either segment or downstream from the junction.

A pond six miles north of Rockport, Texas, on Live Oak Peninsula was Station 3 (Fig. 3). The fish were obtained from Segment A in 1960 and Segment B in 1961. Live samples in 1961 were obtained from Segment A. Segment A was seined in five hauls four times each, two in each direction. Segment B was seined in six hauls two times each, one in each direction. Few mollies were obtained between November, 1960, and March, 1961, due to high waters. The fish were in the bushes adjacent to the pool. It was feared that the effort required to obtain the mollies in the bushes would adversely affect the locality. The water was again high in September, 1961, and fish were obtained in the bushes, as the field work was being discontinued.

The San Marcos River just east of San Marcos was Station 4. The San Marcos fish are not included in much of the discussion because of the irregularity of the collections. The area was collected more regularly after male amazon mollies were found to occur there.

The fish were obtained between Thompson I. and a ditch on the State Fish Hatchery. Mollies are not native to San Marcos, the *M. latipinna* stock probably coming from Louisiana (Brown, 1953) and the *M. formosa* were first noted in 1958 (Drewry, *et al*, 1958). About 16 amazon mollies were accidentally released from the San Marcos State Fish Hatchery into the San Marcos River in 1955. These fish had been obtained at the Lula B. Sams State Fish Hatchery in Olmito, Texas (William H. Brown, personal com-

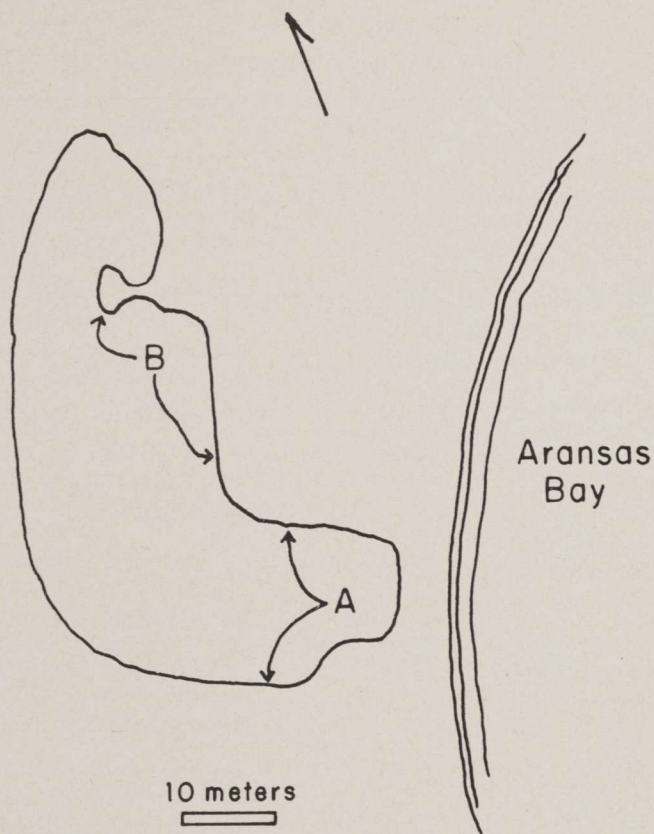


FIG. 3. Sketch map of collection station No. 3 on Live Oak Peninsula, six miles north of Rockport.

munication, 1961). The relative abundance of other fish species at Stations 1-3 is given by Hubbs (1962).

Males of Mollienesia formosa

Phenotypic males of *M. formosa* have been found (Hubbs, *et al.*, 1959). All 31 amazon mollies that have the anal fin modified into (or toward) a gonopodium are listed in Table II.¹ Haskins, *et al.*, (1960) have reported the differentiation of a single *M. spfenops* male in an amazon molly stock tank. As this happenstance has not been repeated and none of our males are

¹ R. R. Miller (personal communication, 1963) reports that two additional males differentiated in a stock tank in Ann Arbor. Both were descendants of fishes collected at the Palo Alto Battlefield. One of the males appears to have been functional in that sibling virgin females isolated with him produced offspring. Unfortunately no information is available concerning the presence or absence of functional paternal chromatin.

TABLE II
List of Phenotypic Males of M. formosa

Date captured	Locality	Disposition	Comments
1) VIII:14:1958	Central Ave.	Preserved	Gonopodium and testis apparently normal; 1108 other <i>M. formosa</i> in collection; 31 mm.
2) VII:25:1959	Central Ave.	Alive	Exposed to 41.5°C during trip to laboratory; sexually excitable; virgin females barren; about 2000 other <i>M. formosa</i> in collection. Died in about 8 months.
3) VII:25:1959	Central Ave.	Alive	Same as Male 2 except higher sex drive; died egg-bound; courted by <i>M. latipinna</i> male.
4) II:20:1960	Central Ave.	Preserved	Mature eggs in ovary, gonopodium normal; 49 mm.; 535 other <i>M. formosa</i> in collection.
5) VI:4:1960	Central Ave.	Preserved	Gonopodium and testis apparently normal; 24 mm.; 367 other <i>M. formosa</i> in collection.
6) VII:24:1960	San Marcos	Alive	Gonopodium normal; died in about one month together with females in tank; lack of courtship probably due to bad water; about 100 other <i>M. formosa</i> in collection.
7) VII:?:1960	San Marcos	Preserved	Collection by Robert Packard; gonopodium and testis superficially normal; histological examination showed ovatestis.
8) IX:17:1960	Lula Sams	Died in transit	Large fish with aberrant gonopodium and no visible gonad; 197 undifferentiated <i>M. formosa</i> in collection.
9) IX:17:1960	Lula Sams	Died in transit	Same as No. 8.
10) X:9:1960	Lula Sams	Died in transit	Large fish with aberrant gonopodium and no visible gonad; 86 mm.; 202 undifferentiated <i>M. formosa</i> in collection.
11) X:9:1960	Lula Sams	Died in transit	Same as No. 10; 56 mm.
12) X:9:1960	Central Ave.	Preserved	Gonopodium and gonad superficially normal; 285 other <i>M. formosa</i> in collection.

TABLE II—(continued)
List of Phenotypic Males of *M. formosa*

Date captured	Locality	Disposition	Comments
13) XI:6:1960	Lula Sams	Preserved	Masculinizing; gonopodium developing; no gonad; 73 mm.; 205 other <i>M. formosa</i> in collection.
14) III:12:1961	Lula Sams	Preserved	Masculinizing; 59 mm.; reduced gonad; large for year class; 74 other <i>M. formosa</i> in collection.
15) III:12:1961	Central Ave.	Alive	Sexually excitable; virgin females barren; 183 other <i>M. formosa</i> in collection.
16) III:25:1961	"San Marcos"	Alive	Transformed in laboratory in cool tank; from San Marcos stock; sexually unexcitable; virgin females barren; died in transit to New York.
17) V:7:1961	Lula Sams	Preserved	Masculinizing, developing gonopodium is slightly anterior; 59 mm.; large for year class; 298 other <i>M. formosa</i> in collection.
18) VI:5:1961	San Marcos	Alive	Sexually excitable; field-captured young that masculinized in laboratory; virgin females barren; can receive transplants from females (Kallman 1962b).
19) VI:5:1961	San Marcos	Alive	Same as No. 18.
20) VI:25:1961	Lula Sams	Preserved	Masculinizing; 62 mm.; gonad reduced; large for year class; 241 undifferentiated <i>M. formosa</i> in collection.
21) VI:25:1961	Lula Sams	Preserved	Masculinizing; 68 mm.; small eggs in gonad; large for year class; 241 other <i>M. formosa</i> in collection.
22) VIII:26:1961	Lula Sams	Alive	Large fish with reduced gonad; no sexual activity; 491 undifferentiated <i>M. formosa</i> in collection.
23) VIII:26:1961	Lula Sams	Alive	Same as No. 22.
24) VIII:26:1961	Lula Sams	Alive	Same as No. 22.
25) VIII:26:1961	Lula Sams	Alive	Same as No. 22.

TABLE II—(continued)
List of Phenotypic Males of M. formosa

Date captured	Locality	Disposition	Comments
26) VIII:26:1961	Lula Sams	Alive	Gonopodium apparently normal; became egg-bound in one month and died.
27) VIII:26:1961	Central Ave.	Preserved	Large fish with reduced gonopodium; 70 mm.; 2,139 other <i>M. formosa</i> in collection.
28) Winter 1961	"Lula Sams"	Alive	Transformed in laboratory from Lula Sams stock; sexually passive, virgin females barren; one of three survivors transferred from 30° to 15°C at birth; died May 15, 1962, apparently normal gonopodium.
29) Winter 1961	"Lula Sams"	Alive	Transformed in laboratory from Lula Sams stock; sexually active; virgin females barren; one of three survivors transferred from 30° to 21°C at birth; died late August, 1962.
30) VII:21:1963	San Marcos	Alive	Gonopodium normal, 39 mm.; 74 undifferentiated <i>M. formosa</i> in collection; developing abdominal swelling typical of egg-bound "males."
31) VII:21:1963	San Marcos	Alive	Gonopodium normal; 41 mm.; 74 undifferentiated <i>M. formosa</i> in collection; moderate sex drive.

notably *M. sphenops*-like, accidental contamination of the stock tank may be involved.

Many of the phenotypic males have gonopodia that lack the gonopodial spines and hood and are otherwise exceedingly aberrant. These mollies (Nos. 8, 9, 10, 11, 22, 23, 24, 25, and 27) are all large (56 + mm.), the gonad is so small that it often cannot be detected, and the anal fin is posteriorly located. Fish 22–25 were maintained in the laboratory for over a month and no gonopodial swinging was observed. All of these attributes indicate that these fish have developed their gonopodia in old age. Although their previous reproductive history is unknown, I suspect that they had been functional females. The nine fish were taken between August 26 and October 9, toward the close of the normal reproductive season.

Five amazon mollies (Nos. 13, 14, 17, 20, and 21) have anal fins that were

apparently changing into gonopodia. It is not possible to ascertain with assurance whether the gonopodia would have become normal; however, as the fish are all large and the anal fin is nearly in the female position, they probably would have also developed aberrant gonopodia.

The other 17 phenotypic male amazon mollies had anteriorly located anal fins with gonopodia similar to that of related species. Nevertheless they often had feminine attributes. For instance, Male 4 was egg-bound when captured. Number 3 had an extremely high sex drive, copulating repeatedly with females in the same aquarium, doing so at about five minute intervals during every observation interval for over two months. When the fish had been in the laboratory for about eight months, the abdominal cavity began to swell and it died, egg-bound, two months later. Male 26, which was apparently normal otherwise, also died egg-bound and a transforming fish, No. 21, had eggs in its gonad.

Male 7 was collected during July, 1960 by Dr. Robert Packard who kindly offered me the opportunity of examining it. Superficially this preserved fish was a normal male since the gonopodium and gonad appeared to be masculine. The gonad was removed, stained and sectioned by Dr. A. G. Jacobson. Obviously it was an ovatestis since many of the tubules contained maturing spermatids, others had ova with yolk, yet others had both (Fig. 4). It is probable that the testicular part would not have become functional.

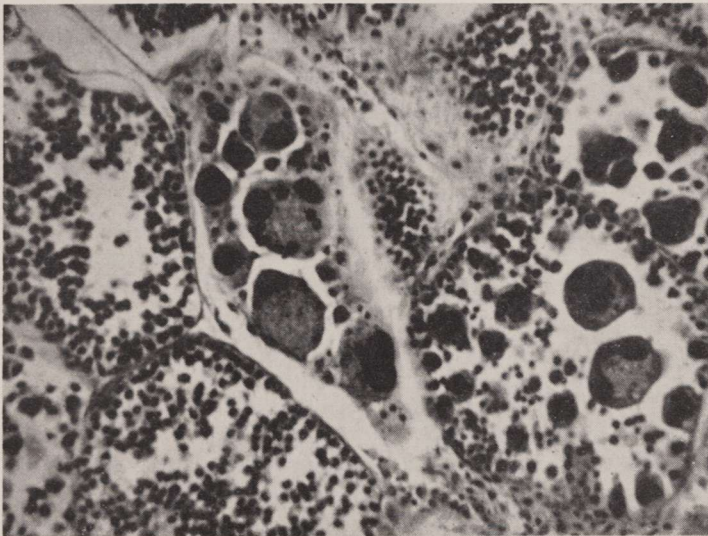


FIG. 4. Photomicrograph of a section of the gonad of amazon molly male 7. Testicular tubules occur at the lower left and elsewhere. An ovarian tubule is in the center. Two tubules to the right contain both developing spermatids and eggs with yolk. None of the sections contained mature sperm or eggs.

Nine apparently normal males (Nos. 2, 3, 15, 16, 18, 19, 28, 29, and 31) were maintained with virgin females for intervals up to 10 months. No young were produced; however, at least some males are partly functional (see footnote 1). Under similar circumstances amazon molly females produce broods at regular intervals when maintained with males of other species. All of the males except Nos. 16 and 28 courted actively and the females were receptive. The females used were derived from stocks obtained at the three amazon molly collection stations as well as stocks maintained by Drs. C. P. Haskins and K. D. Kallman.

Kallman (1962b) reported negative breeding results with Males 18 and 19 which transformed in his laboratory. He was successful in transplanting spleens from amazon molly females (Clone V) into these two males. This information together with the abnormal sexual condition of many males indicates that all of the "males" are genetic females with a variably-developed male phenotype.

It is possible that the development of male attributes is in response to environmental factors. Because the first male had been collected in July (average temperature 28.8°C), high temperature was suspected to be the cause. This hypothesis was abandoned after several thousand amazon mollies were reared at about 37°C and no males developed. Then it was noted that males occur in the spring (Nos. 4 and 5) and that males are relatively abundant in the cool waters at San Marcos (Nos. 6 and 7). Male 5 is 24 mm. long, well above the average size of other young of the year in the same collection. Likewise, Male 1 is 31 mm. long, well above the average size for young of the year in July of 1960 or 1961 (see Fig. 8 for sizes of females). It is probable, therefore, that both were born early in the breeding season. Amazon mollies were then exposed to cooler laboratory conditions. The same stocks that had been tested at 37°C were placed by a window. Male 16, noted on March 25, 1961, had been exposed to cooler conditions than numerous stock mates that included no males. Subsequently newborn *M. formosa* were transferred from 30°C (the temperature at which the mothers were maintained) to 15°C and 21°C. Although mortality was near 99 per cent, in each experiment one of three survivors was masculinized (Males 28 and 29). Such a hypothesis is further supported by the relatively numerous males in the cool waters near San Marcos (six—Nos. 6, 7, 18, 19, 30, and 31—of about 1,000 individuals). The two males transforming in Dr. Kallman's laboratory obviously belong to the first broods of the year. Young were observed first in May, 1961, and Kallman's fish were collected June 5. The two 1963 males appear fully differentiated and are nearly the maximum size for young of the year in the same collection. Although the sample size is very small, it appears likely that exposures to cold temperature during development or shortly after birth is associated with masculinization.

Sex Ratio of *Mollienesia latipinna*

Normal bisexual populations of poeciliid fishes have more females than males. This is due to increased male susceptibility to adverse environmental factors (Krumholz, 1948) and to increased predation on the brilliantly colored and aggressive males (Haskins, *et al.*, 1961). Therefore male frequency may be low enough to reduce the effective breeding population. Selection for an unbalanced sex ratio in sailfin mollies would be most likely in the areas also occupied by the gynogenetic amazon molly. The male frequency might be increased to insure that all mature females would be fertilized. However, if the homospecific females were to be fertilized before the heterospecific females, increasing male sailfin frequency would result in increasing the production of amazon molly young whereas the number of sailfin molly young would remain approximately the same. A reduction in the number of sailfin molly males would accentuate this preference and result in a reduction of the relative number of amazon molly young compared with the number of sailfin molly young.

The sex ratio of the sailfin molly population at Rockport was compared with those at Brownsville (Table III). It is difficult to ascertain with assurance whether a young molly will become a male or female, as some may become phenotypic males and genotypic females and vice versa. Parallel problems have been shown for other poeciliids (Haskins, *et al.*, 1961). Therefore those individuals with a modified anal fin were assumed to be males and those with an unmodified anal fin were assumed to be females. Using those criteria approximately one third of the Rockport fish were males and one

TABLE III
Sex Ratio of Mollienesia latipinna Collected in Nature

	N.	All Fish Per cent		Chi square	N.	Adult Fish Per cent	
		Male				Male	Chi square
Rockport	5990	31.95		469.88	4934	38.79	90.36
Brownsville	3826	12.68			1839	26.37	
1960							
a Rockport	2163	39.71		223.51 a x b	1866	46.03	75.83 a x b
b Central Ave.	1439	16.33		1.40 b x c	833	28.21	4.00 b x c
c Lula Sams	359	18.94		58.31 a x c	305	22.29	60.32 a x c
1961							
a Rockport	3827	27.57		259.30 a x b	3068	34.38	14.16 a x b
b Central Ave.	1871	8.93		0.07 b x c	627	26.63	1.39 b x c
c Lula Sams	157	9.55		24.96 a x c	74	20.27	6.14 a x c

eighth of those from Brownsville were males, a difference which is highly significant. If the figures are broken down by year, the differences remain significant; and the two Brownsville populations showed similar sex ratios.

The above figures include many immature males as females. It is possible to reduce this error by excluding all immature fish. The smallest fish with mature ova were 21 mm. at Rockport and 31 mm. at Brownsville. I have assumed that all smaller undifferentiated ones were immature and that those larger are females (Table III). This should bias the data in favor of increased frequency of females at Rockport because undifferentiated Rockport fish between 21 and 30 mm. were considered female whereas those of equal size from Brownsville were considered immature. However, the larger percentage of females at Brownsville persisted so there is little doubt that the natural populations in Brownsville contained fewer males. The marginal significance of the difference between 1961 Rockport and Lula Sams samples was probably due to the small sample at Lula Sams.

The general decrease in male frequency of the total sample in 1961 was due in part to the absence of collections during the fall when more young of the year would have differentiated. The difference in adults may have been from the same cause; however, it is also possible that the more severe weather during the winter of 1959-60 stimulated the differentiation of more males.

It is possible that differences in the collection localities and the relative sampling efficiency in ponds and ditches may have differentially affected the sex ratio of the samples. To test this hypothesis similar comparisons were made on *Cyprinodon variegatus*, a fish that occupies the same general habitat and also has territorial males and essentially nonterritorial females. The males comprised from 44 to 60 per cent of the adults at Brownsville and 42 to 56 per cent in Rockport. There is no apparent difference between these sex ratios and certainly nothing of a magnitude sufficient to explain the difference in molly sex ratios.

The different molly sex ratios may be due to genetic or environmental factors. The prolonged hot weather at Brownsville might result in increased mortality which might affect males more than females and the colder winters in Rockport might stimulate increased masculinization there. Likewise the increased number of females might increase the courtship activity of Brownsville males. It is probable that courting males are more vulnerable to predation than non-courting males. Predation may play a role as the number of males in the adult population is lowest at Lula Sams, where a flourishing largemouth bass population (*Micropterus salmoides*) is resident. The only fish collected at Central Avenue that would be a possible predator on adult mollies is *Fundulus grandis*, a species which occurs at all three localities. Differential predation seems significant in comparing the sex ratio of the

July 23, 1961, collection from Segment C with the others from Station 1 on the other side of the culvert. The July collection was 5.5 per cent male and had many predators collected in every seine haul. Differential predation does not account for the large number of males at Rockport as several other potential predators were in residence (*Opsanus*, *Cynoscion*, *Fundulus similis*, *Paralichthys*, etc.).

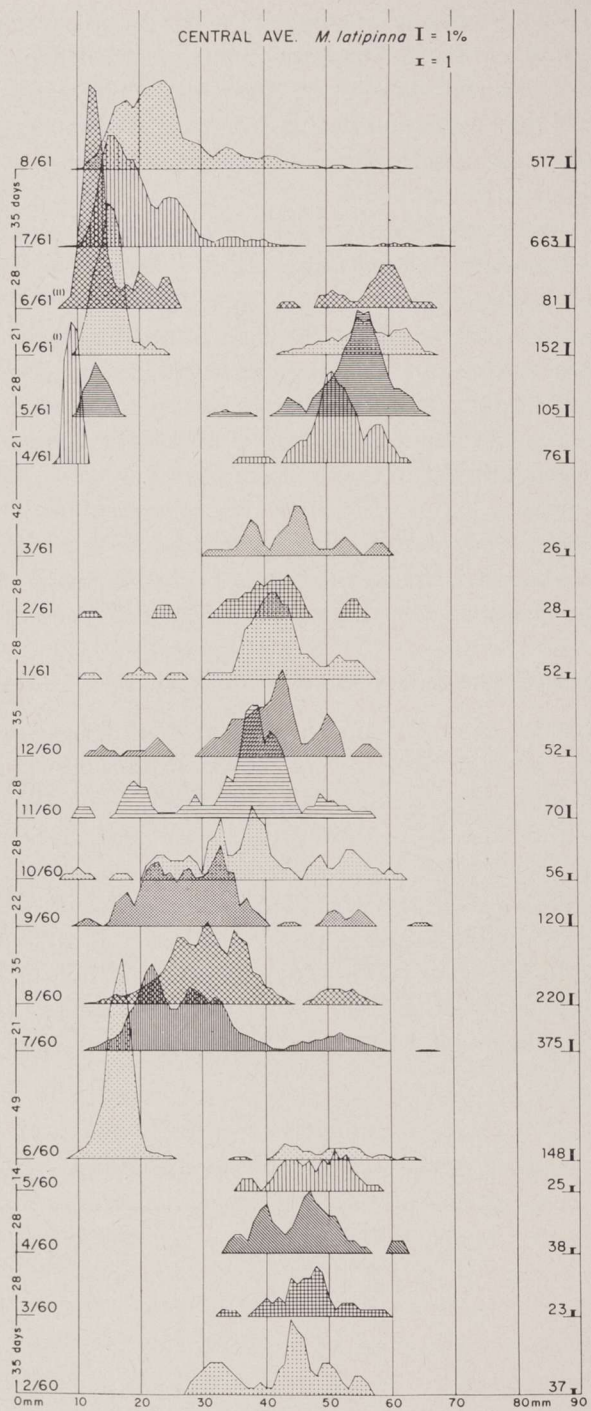
Several fish were reared in the laboratory to confirm the genetic basis of the sex ratio differences. Female sailfins from Rockport and Lula Sams were brought to Austin. The resulting broods were placed in four pairs of aquaria. Each aquarium contained several broods, and a Rockport aquarium was placed with one containing offspring from Lula Sams. The fish were preserved six months later (after the first broods were produced). The males were more frequent in each of the four pairs of Rockport aquaria and the differences were significant in two of the pairs (Table IV). The two with no statistical significance had 8 or 13 survivors in one aquarium. An additional group of young reared in individual pans had 30 per cent males in the Brownsville stock and 40 per cent males in an allopatric stock from Port Aransas, Texas; however, the differences are not of statistical significance.

Reproductive Season and Growth Rate of M. latipinna

Poeciliid fishes are often the most abundant small fishes in shallow tropical coastal waters. They are probably of considerable importance in the food chains there. A number of workers (Gunter, 1945; Reid, 1954, 1955; Strawn, 1958; Springer and McErlean, 1962, etc.) have noted that most of the small tropical coastal fishes have prolonged summer breeding and few fish live through their second winter. Kirby (1955), the only one who worked with *M. latipinna*, was unable to ascertain size classes and attributed this to the prolonged breeding season. He did not separate the sexes, however, and the males complicate analyses as their growth slows or ceases after sexual maturity. Moreover, he pooled several sub-localities and dates, which would

TABLE IV
Sex Ratio of Mollienesia latipinna Raised in the Laboratory

	Brownsville		Rockport		Chi square
	N.	Per cent Male	N.	Per cent Male	
Tank A + A'	72	11.1	66	31.8	8.82
Tank B + B'	8	12.5	55	41.8	0.24
Tank C + C'	141	12.8	13	23.1	1.02
Tank D + D'	55	23.6	73	46.6	7.11
Total	276	14.5	207	39.1	38.12



also make an analysis more difficult. I have plotted size classes for females only and kept every collection separate, and can follow the year classes. The validity of these year classes is supported by the presence of an annulus in fish considered to have over-wintered and its absence in those that have not.

Size distribution data are presented in Fig. 5 for *M. latipinna* females from Station 2 (Central Avenue ditch). Few adults were present in early 1960. The first young appeared in June, which correlates well with the first occurrence of pregnant females in the April collection (Fig. 11). Growth is rapid until July, when some young exceeded 40 mm., and continues to be relatively rapid until October. It slows until February or March and then is again relatively rapid until the last sample. Small fish were found until February, but they were probably born before November, as no pregnant females occurred in the November, December, and January collections. The major difference between the fall and spring collections is the absence of small fish in the spring. I attribute this to the younger (smaller) fish in the year class catching up with the older (larger) ones. The 1959 size class is difficult to follow after October 1960 and appears to have been lost during that winter. The 1961 year class appears first in May, following the first detection of pregnant females in March. These young grow rapidly and begin to obscure the 1960 year class by August when the collections were terminated.

The difference in the first appearance of young in 1960 and 1961 is probably due to the colder weather in 1960 (Fig. 6). The temperature difference is most notable after the seventh week of the year at which time the average temperature was over 21°C in 1961 and below 14°C in 1960. Weekly average temperatures in excess of 21°C after the middle of January did not occur in 1960 until the fifteenth week. Similarly, *Micropterus salmoides* bred in early January, 1961, and late January, 1960, at Lula Sams (Houston Maples, personal communication, 1961).

The less severe winter in 1961 may also have resulted in a reduced mortality. Four collections from February through May, 1960, contained 158 adult *M. latipinna* compared to four collections at corresponding intervals in 1961 which had 286 adults. This 80 per cent increase in population density occurred despite extensive sampling (predation) during the preced-

FIG. 5. Size distribution of females of *M. latipinna* from the Central Avenue ditch at each collection date between February, 1960, and August, 1961. The standard length in millimeters is listed on the ordinate. To reduce recording preference, the data are presented as three point rounded curves. The vertical difference between the base lines reflects the time between collections. The number of specimens involved is given at the right. If more than 66 specimens were present, the percentage of the total population is plotted. If fewer specimens were present, the individual figures are used, which reduces the prominence of the few fish in the small collections.

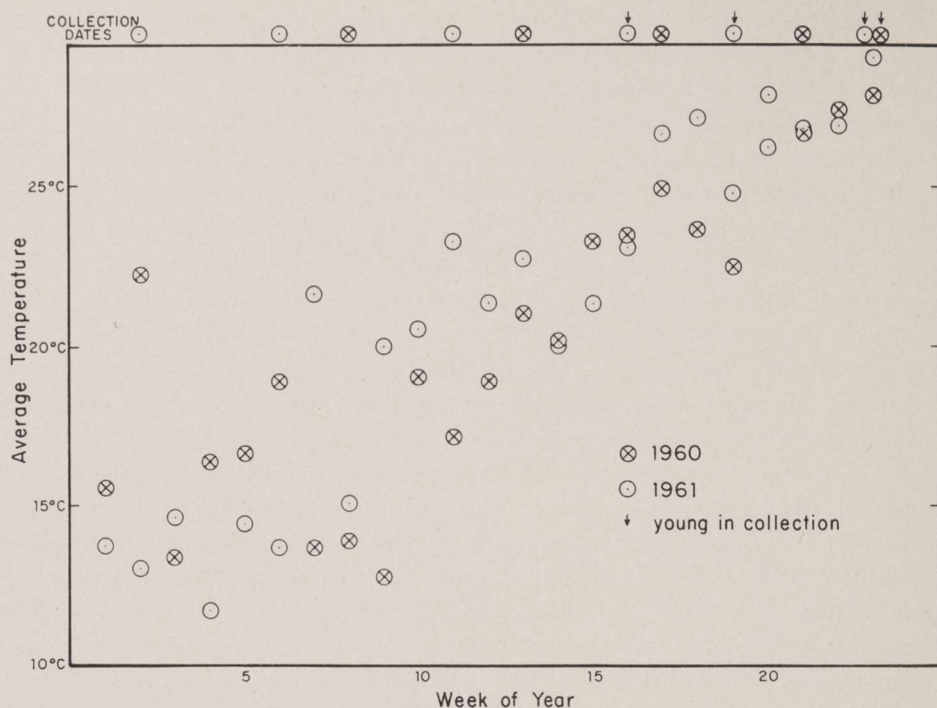


FIG. 6. Average weekly temperatures at Brownsville for the first weeks of 1960 and 1961, taken from the data published by the U.S. weather station at Brownsville. The weeks in which the collections were made are listed at the top of the figure, and those including young of the year are designated by arrows.

ing fall. The population size in July and August, 1961, was twice that at comparable times in 1960, which may reflect the increased adult population and/or longer breeding season.

Size distribution data for *M. latipinna* females from Rockport (Fig. 7) resemble those from Central Avenue. The first young appeared in July, 1960, and June, 1961. The delay probably reflects the more northern latitude of the Rockport locality ($28^{\circ} 06' N.$) compared with the Central Avenue locality ($25^{\circ} 54' N.$). The difference between the two years is probably based on the mild winter weather in 1961. More adults were taken in April and May of 1961 than in the same months of 1960, which may reflect the warm winter. The virtual absence of mollies at Rockport on June 2, 1962, after a winter in which numerous cold records were set supports this hypothesis, and mollies also were difficult to obtain in Brownsville the following day. The increased number of young probably reflects the increased number of adults and longer breeding season. The annual nature of the Rockport population is more pronounced. This is probably due to the shorter breeding season which increases the separation between year classes and to increased winter mortality, which reduces the probability of a fish living through two winters.

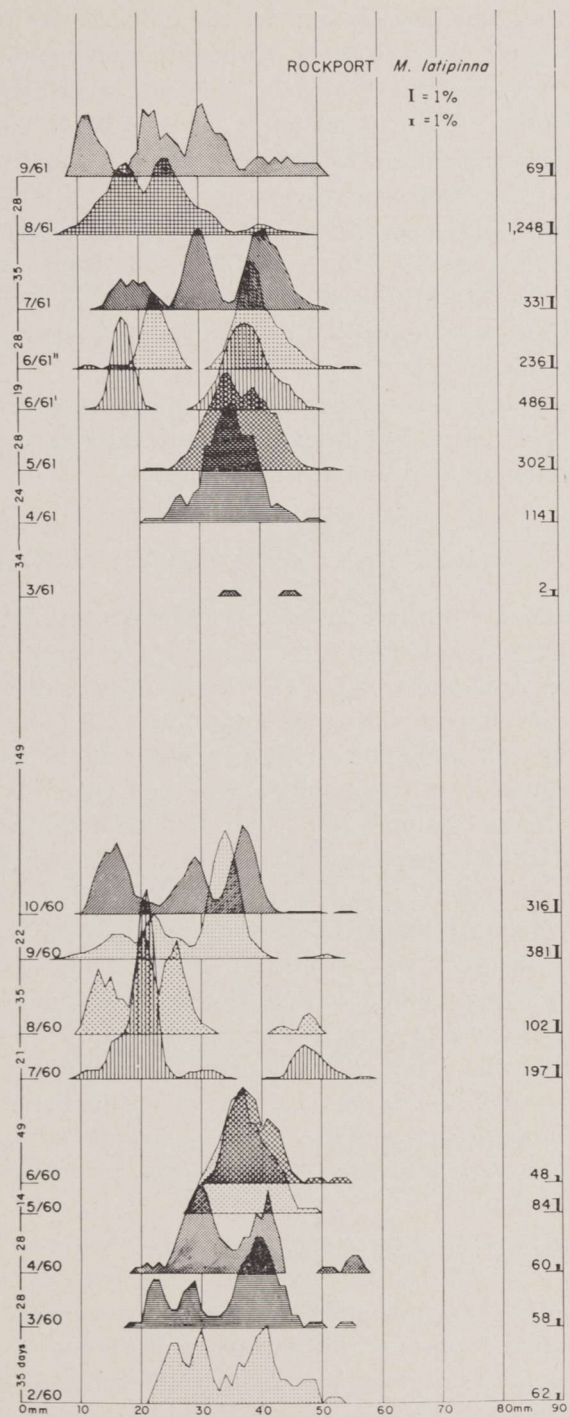


FIG. 7. Size distribution of females of *M. latipinna* from the pond north of Rockport. The other information is as listed for Fig. 5.

The colder weather may also account for the reduced size range. The 1960 year class grew rapidly until October. Although winter data are absent, the 1960 year class in April is essentially the same size as the larger individuals the previous October. There is an apparent over-winter increase in year class uniformity as occurred in Central Avenue. Growth resumed again in May or June and the 1960 year class was virtually absent in September.

The young of the year size class in late summer and early fall collections often has two or three peaks. This probably results from a synchronization of brood production. Multiple peaks are less notable in Brownsville, which may reflect increased synchrony of Rockport females resulting from a more abrupt seasonal change.

Size distribution data are not presented for sailfins from Lula Sams because too few were collected during the months after dredging. Otherwise the data are similar to those from Central Avenue.

Growth Rate and Reproductive Season of M. formosa

The growth rate and reproductive season of the amazon molly in nature has not been studied previously. Although males would scarcely affect the analyses, they are excluded because they cease growth at sexual maturity. Size group analyses may provide some insight into the biology of the gynogenetic species as well as the interactions between the two species.

Size group analyses of amazon mollies from the Central Avenue Ditch are similar to those for the sailfin molly (Fig. 8). The first young in the 1960 sample appeared in June, grew rapidly, and some exceeded 40 mm. in length by July. Growth continued until October after which it slowed until February and March, when the 1960 year class resumed growth. The peak for the largest young of the year was 15 mm. in June, 38 mm. July, 40–41 mm. August, 41 mm. (?) September, 45 mm. October, 43–45 mm. November, 45 mm. December, 46 (?) mm. January, 47 mm. February, 52 mm. March, 53 mm. April, and 61 mm. May. The relatively more rapid growth in warm months is easily traced in Fig. 8. The first young appeared in April 1961, which was undoubtedly due to the same factors which influenced early sailfin broods that year. The growth was less rapid in 1961 but some young exceeded 40 mm. by July.

Winter and spring collection in 1960 contained two size classes of adults. These probably represent the 1958 and 1959 year class as the larger fish in the February sample have one annulus whereas the smaller ones have none. The new winter mark is present in the April collection so that the fish have two and one year marks respectively. Some of the fish have finished annulus formation in March, and others have not. There is a distinct difference in the growth rates of the one and two year old year classes. The one year old mode changed from 40 mm. in February to 55 mm. in August and September. Dur-

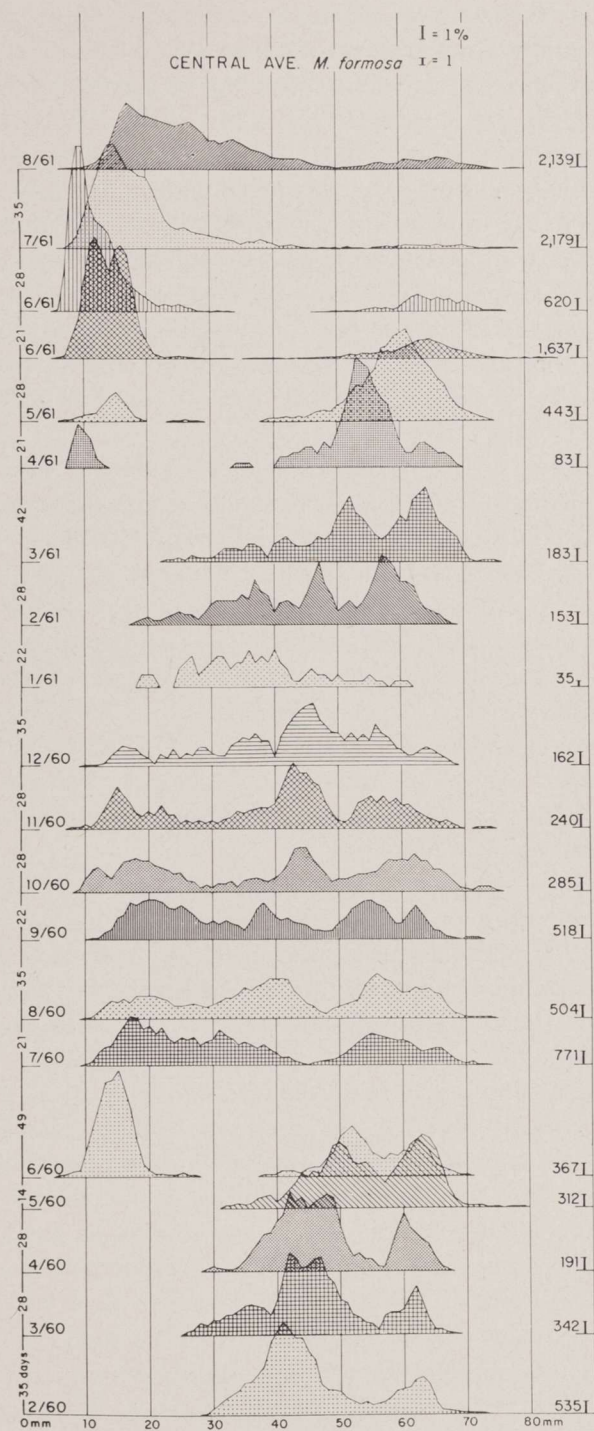


FIG. 8. Size distribution of females of *M. formosa* from the Central Avenue ditch. The other information is as listed for Fig. 5.

ing the same time the two year old mode remained at approximately 63 mm. In 1960, large sailfins from Central Avenue were also often two years old; therefore, the apparent interspecific difference in survival of two year olds is an artifact of sample size in sailfins. Only a few of the 1959 year class of amazons survived until the spring of 1961. The smaller numbers probably reflect increased predation resulting from this investigation. In early 1961 the yearlings had modes in excess of 50 mm., nearly 10 mm. longer than in 1960. This may be due to the warmer winter or to reduced competition because of the sampling. As both samples of *M. latipinna* were also longer in the spring of 1961 (Fig. 5), and the sailfin sample sizes were larger then, competition is a less likely factor than the increased growing season. The differences between the yearling and two year old modes in 1961 is less than in 1960. This can also be explained by the milder winter.

Many more young fish were produced in 1961 than in 1960. This cannot result from an increased breeding population as the February, March and April, 1961, collections contained fewer individuals than did those in comparable months in 1960. The three to four-fold increase in population size in summer is due to the increased number of young. It is probable that the number of young produced is a reflection of the *M. latipinna* population density, or in effect the number of available males of that species. The sailfin population was about 80 per cent larger in 1961 than in 1960. The peaks for the amazon molly young of the year during the start of the breeding season are much lower than those for the sailfins. This suggests that the sailfin males first copulate with sailfin females, and when these are pregnant they court and copulate with amazon females.

Size group plots of amazon mollies collected at Lula Sams State Fish Hatchery resemble those of the same species from Central Avenue (Fig. 9). Growth of the 1960 year class was rapid in the summer, slow in winter, and resumed in the spring. Young were produced earlier in 1961 than in 1960, probably for the reason discussed above. Likewise, yearlings were larger in 1961 than in 1960.

The two samples differed in some details. The later appearance of young at the hatchery compared with Central Avenue in both years is probably because the hatchery seining station did not include shallow habitats favored by young mollies. This is supported by the first occurrence of young having modes at 20 mm. or more whereas many fish 10 mm. or less have been collected at Central Avenue. Laboratory-produced newly born young from Lula Sams stocks are 10 mm. or less in standard length. Two year old fish were scarce at all times. This can be attributed to the presence at the Lula Sams Hatchery of a flourishing largemouth bass population which would prey extensively on mollies. All predatory species known to be at Central Avenue also have been obtained in large numbers at the hatchery. It is possible that sampling may have had a greater effect on this population as the sample size

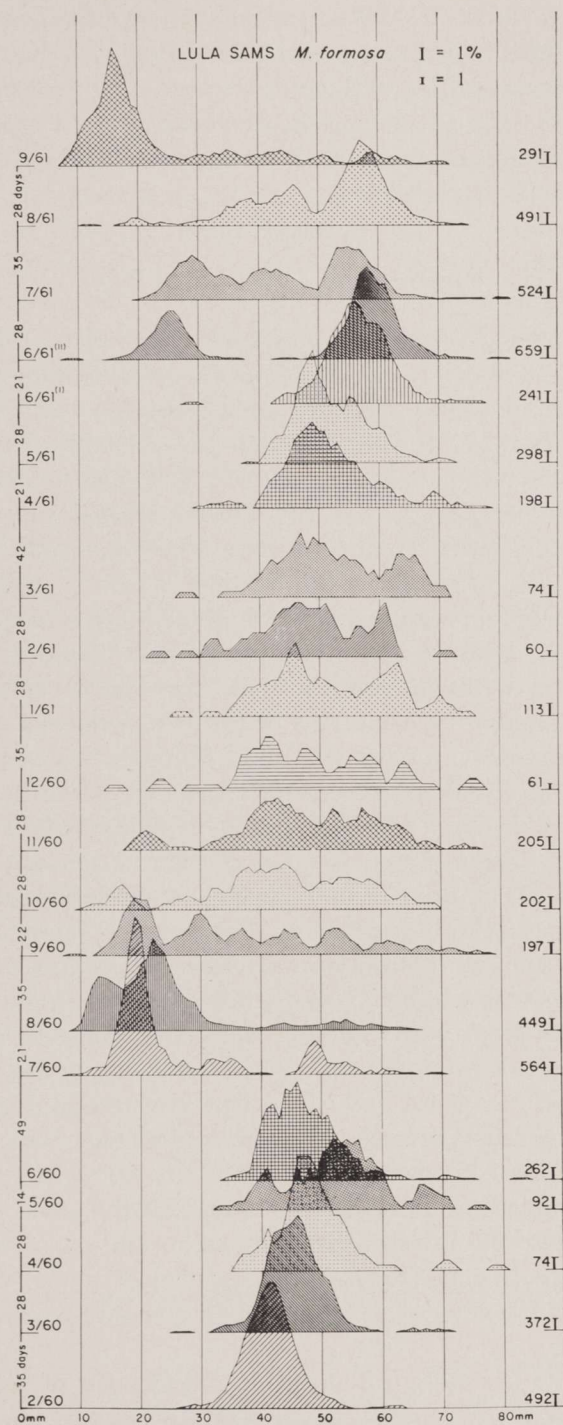


FIG. 9. Size distribution of females of *M. formosa* from Lula Sams State Fish Hatchery. The other information is as listed for Fig. 5.

was drastically reduced in 1960 even before the ditch was dredged. As a result the July, 1961, collection was obtained from an adjacent locality (C) and the collection from the following September included fish from both localities B and C. The presence of many young fish at that time could have resulted from the changes following Hurricane Carla, as much of the flooded area that was seined was shallow.

Ovulation and Pregnancy Rates

Most closely related vertebrate species occupy similar habitats. Interspecific competition could involve a number of factors including food and space. All recent workers have considered that the amazon and sailfin mollies are closely related. It is highly probable that the amazon molly resulted from hybridization between the sailfin molly, *M. latipinna*, and its near relative *M. mexicana*. A hybrid between two closely related species would have habitat requirements very similar to those of the parental species, and observations in Brownsville support this assumption. Both species are primarily herbivorous, feeding on periphyton; both prefer warm shallow pools, and the young are born at about the same time and grow at similar rates. Equal-sized females produced approximately the same number of young (see Figs. 19 and 20 for comparable presentations of the two species at Central Avenue recalling that amazons are somewhat more elongate and should not contain as many young as an equally-long sailfin female). There is a tendency for sailfins to be more common among emergent vegetation and cooler water, but the difference involves a change of no more than 10 per cent in relative density. Under such circumstances interspecific competition could approach the intensity of intraspecific competition. The relative population density of females in any year class should approximate the production of young that year. If so, *M. formosa* would have a distinct advantage in that all (or almost all) of the adults are female. Some of the sailfins must be males, and that fraction would result in an equivalent reduction in number of offspring. If the sailfin sex ratio were 1:1 and other factors were equal, a mixed population with equal numbers of each species would include these percentages of sailfins in successive generations: 33.3, 20, 11.1, 5.9, 3, 1.5, 0.78, 0.39, 0.19, 0.098, etc. This could result in so few sailfins that the sexes would not meet, which would cause the extermination of sailfins and consequently the amazons. Although these conditions are not equaled in nature, differences such as an increased percentage of bisexual females would merely prolong the extermination time.

Some means of increasing the relative recruitment of sailfin young is a logical way to counteract the all-female reproductive advantages of the amazon molly. Any amazon population that was able to equal this relative re-

cruitment gain would cause its own extermination; therefore, the advantage should be retained. The increased recruitment of sailfin mollies could reflect a longer reproductive season or an increased preference for homospecific copulation by the males. Analysis of the ovulation rates of the two species does not indicate an increased availability of sailfin females (Fig. 10). On the contrary, amazon molly females ovulated earlier at Brownsville than did the sailfin females. The end of the ovulation period is more nearly equal and appears to be slightly later in amazon mollies. The later appearance of ovulation at Rockport probably is due to the more northern collection locality.

Analysis of the pregnancy ratios in the two species provides clear evidence for a reproductive advantage by sailfin females (Fig. 11). September, 1960, was the only time the large adult female sailfins collected at Brownsville had a lower pregnancy percentage than did the amazons from the same localities. These collections contained only six large sailfin females, none of which was pregnant, and 31 young ovulated females, 21 of which were pregnant. This fact along with the high incidence of pregnancy during the adjacent months indicates that sampling error may be involved during September, 1960.

The low pregnancy percentage for sailfins from Rockport in June and July, 1961, is difficult to evaluate. It is possible that it reflects some sort of stress reaction (Christian, 1959) as the population was very dense. It is more probable, however, that most females were in synchrony and the samples were

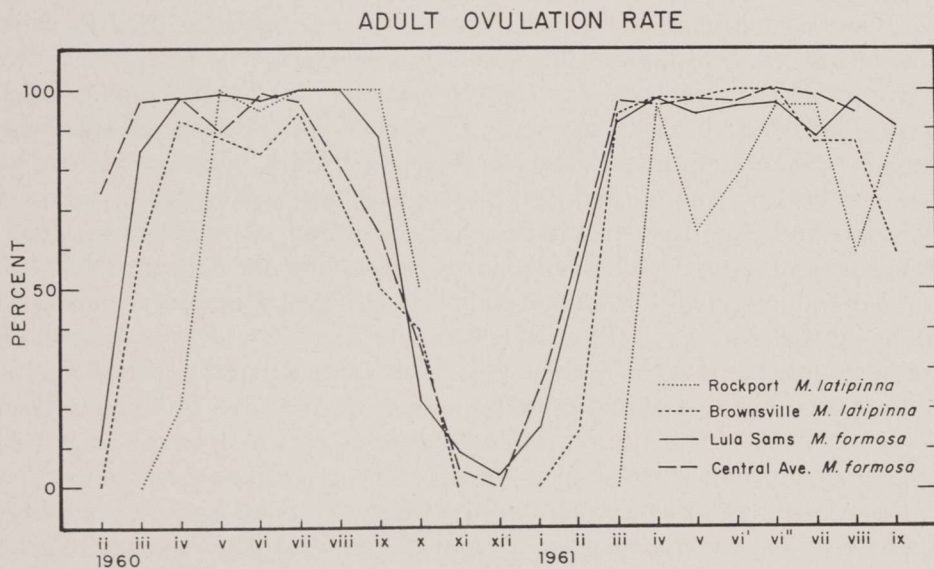


FIG. 10. Percentage of the adult females with mature eggs obtained in each collection. Only females 45mm. and 35mm. or larger are tabulated for the Brownsville and Rockport stations respectively. The Roman numerals refer to the months in which the collections were made. See Table I for the exact dates.

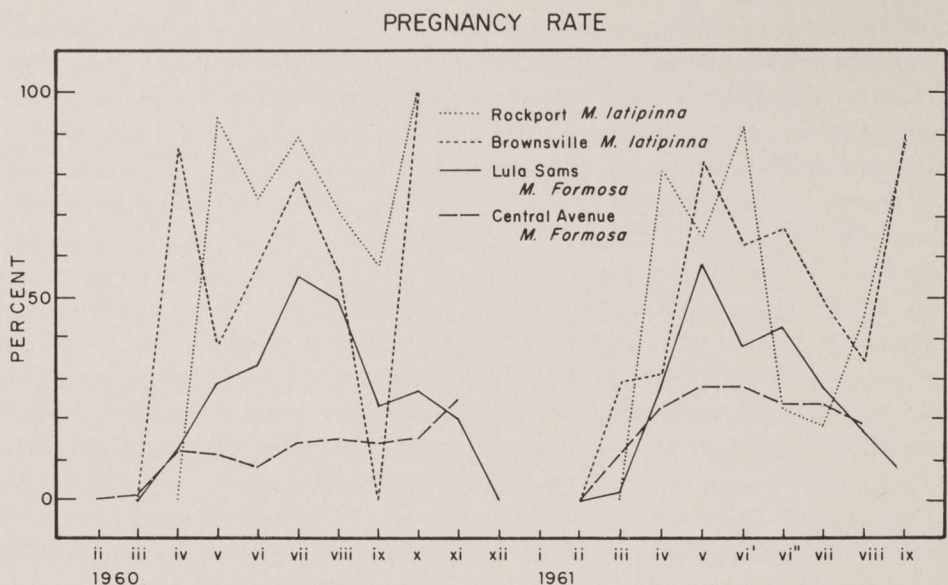


FIG. 11. Percentage of the ovulated females that contain one or more embryos with pigmented eyes. The other information is as listed for Fig. 10.

taken between the broods. The females did contain sperm as nearly 70 per cent of the females in a live sample obtained in July, 1961, produced broods (see below) and most of the broods were born after the broods of Brownsville females of both species collected the following day.

The pregnancy percentages of amazon mollies from Lula Sams is much higher than that for amazons from Central Avenue. Amazon mollies are abundant in both localities; however, there is a distinct difference in the relative numbers of amazon and sailfin mollies at the two localities. Between February and May, 1960, before Lula Sams was dredged, 1,030 amazons and 244 sailfins were collected at Lula Sams. The comparable collections at Central Avenue contained 1,381 amazons and 158 sailfins. The relative density of sailfins at that time was 1:4.2 at Lula Sams and 1:8.7 at Central Avenue. Collections subsequent to the dredging exhibited more equal ratios between the two species; however, samples taken from Segment C at Lula Sams often contained half to one quarter as many sailfins as amazons. An increased pregnancy percentage of the gynogenetic females accompanying an increase in the relative population density of the bisexual species is in accord with a hypothesis that the gynogenetic females are courted after bisexual females are pregnant.

The pregnancy percentages of amazon mollies from Central Avenue were higher in 1961 than in 1960. The relative density of sailfins was also higher in 1961 than in 1960, being approximately 1:1 in January and April and 1:4

in February and March. Therefore the effects of relative population densities are approximately the same whether synchronic populations are contrasted or the same population is compared at different times.

Pregnancy ratios undoubtedly correlate with production of young as the appearance of young in the natural populations follows the capture of pregnant females (compare Fig. 11 with Figs. 5, 7, 8, and 9). Living adult females were returned to the laboratory in late 1960 and early 1961 and isolated in gallon jars to further test this logical hypothesis. Those females that died within one week after isolation without producing young were excluded from consideration. The mortalities were similar in all samples obtained at any given time. Although the data obtained are often not statistically significant, the patterns are remarkably similar to those obtained from the field samples. Seventeen (nine Lula Sams and eight Central Avenue) amazon mollies were isolated from the December collection. None had produced offspring by the time this series terminated at the January collection date. Likewise none of the nineteen (fourteen Lula Sams and five Central Avenue) amazon mollies obtained in January had any broods before the February collection. Twenty amazon mollies (equally divided between the two localities) were obtained in February. One from Central Avenue produced a brood on March 11. Young were not obtained there the next day, but a few females were pregnant. It is probable that the laboratory brood was chronologically early because the females had been maintained at 25° C. The March live sample contained 11 female amazons from each Brownsville locality. Three broods were produced, one from Central Avenue and two from Lula Sams. The 19 females (15 Lula Sams and four Central Avenue) in the April collection produced four broods (all Lula Sams). The above data indicate a slow start to the reproductive season that correlates with the relatively slow increase in amazon molly pregnancy ratios.

Five of the remaining six live samples were primarily designed to contrast sailfins with amazons. In the other, obtained on June 25, 1961, 20 amazons from Central Avenue produced 11 broods (1:0.55), 35 amazons from Lula Sams produced 23 broods (1:0.66), and ten sailfins produced eight broods (1:0.80). Preserved sailfins in the same collection had a higher pregnancy percentage than amazons and the *M. formosa* females were more likely to be pregnant at Lula Sams than at Central Avenue.

The May, 1961, collection contained 33 Brownsville amazons, 15 of which produced broods (1:0.45), and nine Brownsville sailfins, five of which produced broods (1:0.55). A slightly higher mortality of sailfins (3 of 4 non-productive females) may account for a productivity similar to the amazons, in which only 8 of 18 nonproductive females died after the first week. The June 4 and 5, 1961, collection contained 23 females of each species from Brownsville. Eight of the sailfins and five of the amazons produced broods

within three weeks. Seven of the nine sailfins from Rockport produced broods.

The July and August collections were maintained for several months, therefore the data are deemed to be more significant. The July collection contained 32 amazons from Lula Sams. Fourteen produced broods (1:0.44) and three produced a second brood (1:0.09). Twenty-four of the 31 Lula Sams sailfins produced broods (1:0.77), fifteen produced a second (1:0.46), and one produced a third (1:0.03). Twenty-three of the 33 sailfins from Rockport produced broods (1:0.69), 16 produced two (1:0.48), two produced a third (1:0.06), and one produced a fourth (1:0.03). The productivity of the amazons is distinctly below that of the sailfins. The August collections were infested with furunculosis, so few females produced a second brood. All fish were from Brownsville. Thirty of the 67 amazons produced a brood and 57 of the 69 sailfins did so. A collection from Segment C at Lula Sams made in June, 1962, contained 41 amazons, three of which produced broods (1:0.07) and 52 sailfins, 34 of which produced broods (1:0.65). These females were so large that the males might have been able to discriminate with an increased efficiency.

Some of the females in the July collection from Rockport produced their first brood long after isolation. One female produced its first brood on September 2 and two produced their first broods on November 5. Another female from the same locality had produced its second brood on September 7 and its third on October 11. Another female produced its second brood on September 15, its third on October 22, and its fourth on December 3.

Courtship Preference

The difference between the pregnancy rates of sailfin and amazon mollies at the same locality must reflect more intensive courting of sailfin females. Courtship preference tests were run using the techniques described in Hubbs and Delco (1960 and 1962). All females were obtained in Brownsville and held until brood production ceased. Equal-sized females were then presented to a series of wild-caught males. The males thrust at the sailfin females 170 times and at the amazon females 55 times (24.4 per cent). They also nibbled the sailfin females 93 times and the amazons 48 times (34.9 per cent). Both differences have a probability of less than 0.01 of being by chance. Tests with laboratory reared males were similar: males directed 25.0 per cent of the thrusts and 36.8 per cent of the nibbles toward the amazons. Similar comparisons using allopatric males are statistically significant but the discrimination appears less well developed as the amazon females were thrust 39.7 per cent and nibbled 38.4 per cent of the time. The lower discrimination of allopatric males is also statistically significant at the 0.01 level.

Partial Pregnancy

Many females contained some eggs with embryos and others without. These partially pregnant females probably are the result of exhaustion of sperm supply in a female. Under normal circumstances females would be inseminated with such frequency that each would have sufficient sperm for each group of eggs. The abnormal sex ratios of Brownsville populations could reduce the incidence of copulations with individual females so that several broods might be produced between inseminations. Under these circumstances some females would not have enough sperm for a given group of eggs. As courtship is most often directed toward sailfin females, the incidence of partially pregnant females should be greatest among the amazons. This hypothesis appears to be valid as 44.0 per cent (Lula Sams) and 36.5 per cent (Central Avenue) of the pregnant amazon molly females were partially pregnant whereas only 16.3 per cent of the pregnant Brownsville sailfins were. The differences have less than 0.001 probability of being by chance. The abnormal sex ratio in Brownsville may have an effect on sailfins as only 11.8 per cent of the Rockport sailfins are partially pregnant. The partially pregnant females also differ in the per cent of eggs that contain embryos: 59.9 per cent for Lula Sams amazons, 57.4 per cent for Central Avenue amazons, 68.2 per cent for Brownsville sailfins, and 81.2 per cent for Rockport sailfins. These data record an effective reduction of the amazon molly reproductive potential. As stated above, I believe that it reflects a differential insemination frequency. It also may involve a difference in the number of spermatophores transferred during each copulation, which would be expected because the males are less avid in their courtship of amazon females. Both alternatives are probably involved and the evolutionary phenomena are primarily concerned with selection of sailfin males. The time of survival of sperm in the females may also be involved; however, this is less likely as females of both species produce approximately the same number of broods when isolated with sailfin males. Moreover, the evolutionary phenomena could involve natural selection of the amazon females. This is unlikely as the females that were most affected would produce fewer offspring and their genotypes would be selected against.

The live samples brought to the laboratory may have included partially pregnant females. The mollies of both species collected in July from Brownsville were of approximately equal size, most being 50 to 60 mm. long. First broods of females of that length often include 40 to 60 young. The notable exceptions were mostly amazons which produced: 7 (57 mm. long), 3 (59 mm.), 3 (58 mm.), 10 (53 mm.), 15 (55 mm.), and 26 (59 mm.) young. None of these produced a second brood. The lowest brood numbers of equal-sized sailfins were 20 (52 mm.), and 29 (51 mm.), and both of these produced a second brood. Therefore, the six females most suspect of being partially

pregnant are all amazons. The incidence of second broods in the same collection, reported above, also suggests that amazons (21 per cent) contained fewer sperm than did sailfins (62 per cent). Moreover, the number of young in the second broods compared with the first broods of the same individual females tends to be more reduced in amazons (45.7 per cent of first broods) than in sailfins (54.9 per cent).

Superfoetation in Mollienesia

Superfoetation occurs when a female has two or more broods simultaneously in the ovary. This phenomenon has been noted in a number of poeciliid fishes; however, it has not been noted previously in *Mollienesia* (Turner, 1937).

The July, 1961, collection contained six amazon females that had eggs in at least two developmental stages. One had 46 eggs containing embryos with pigmented eyes, a well developed nerve cord, and skin pigmentation; she also had two embryos in which the nerve cord was invaginating. Three had 56, 53, and 45 embryos that lacked the yolk sac and were ready to be born; and also had one, one, and two embryos respectively that had just finished nerve cord formation. Two had 55 and 50 embryos ready to be born and four and three embryos respectively with large yolk sacs and slight skin pigmentation.

Two amazon females in the June, 1961 live sample seem to have held two simultaneous broods. One produced three young on June 29 and 47 young on July 7. The other produced 84 young on June 28 and 66 young on July 6. The females were checked daily in the intervening days and it is inconceivable that 47 and 66 young could have been overlooked for more than one week. The occasional discovery of one to three young a week or more after a brood was produced may also have resulted from superfoetation; however, it is equally possible that the young were overlooked.

The cause of superfoetation in mollies probably differs from that of other poeciliids, in which groups of eggs mature and are fertilized while preceding broods have not yet been born. In mollies, large groups of eggs mature simultaneously, typically develop together, and young are born before yolk deposition and fertilization of the next eggs occurs. The Brownsville populations differ from most poeciliid fish populations in having an excessive number of females. Insemination in such populations would be infrequent. If a female had used up most of her stored sperm when a brood was produced, only a fraction of the next group of eggs could be fertilized and the remainder would remain as mature eggs. Such a fish would be a partially pregnant female. If she were inseminated at this time, she could hold two broods simultaneously or exhibit superfoetation. This happenstance should be most common in the amazon molly.

*Relationship Between Number of Ovulated Eggs and
The Occurrence of Pregnancy*

Increased pregnancy rate of sailfin mollies and increased frequency of partial pregnancy of amazon mollies accounts for much of the differential recruitment of the two species. The rest is due to a difference in the relative probability of pregnancy of females with few and many eggs.

The number of eggs in nonpregnant, partially pregnant, and entirely pregnant females collected from Rockport are given in Table V. In nine of the 12 collections that contained both nonpregnant and pregnant ovulated females, the pregnant females have a higher average egg complement. The difference in all but one of the samples has a probability of less than 0.01 of being by chance. On the other hand, in three of the collections there is a higher average number of eggs in the nonpregnant ovulated females. The difference in two samples, June 24 and July 22, 1961, is far from significant and could be due to chance, or it could be associated with the low pregnancy percentages that occurred in the same collections (Fig. 10). Nonpregnant females in the June 5, 1960, collection contained significantly more eggs than did their pregnant counterparts. I believe that this results from a prior preference for the more fecund females. The most fecund females might be

TABLE V

*Average number of eggs in ovulated female sailfins collected at various times in the
Rockport Station. See Table I for the exact collection dates. The statistical
computations were carried out using a comparison of two samples
as described by Snedecor (1946).*

Months	Not Pregnant	Partially Pregnant	Entirely Pregnant	t	n	p>
1960						
April	24.4					
May	26.0	30.1	31.1	3.37	44	0.01
June	40.6		30.0	7.87	46	0.001
July	41.5		108.6	26.20	43	0.001
August	16.6		52.6	26.31	18	0.001
September	22.6	17.0	38.1	7.89	245	0.001
October			18.2			
1961						
April	21.4		37.6	13.43	76	0.001
May	17.0	33.5	28.5	7.17	163	0.001
June I	12.2	19.7	19.9	5.12	228	0.001
June II	32.2	36.0	28.6	1.89	144	0.1
July	32.8	35.0	31.6	0.47	205	0.7
August	12.7	15.9	15.4	3.10	320	0.01
September	14.0	30.0	32.6	0.88	11	0.5

tabulated as nonpregnant if these females had produced their first broods and were initiating development of the next brood at the sample time. Multiple peaks in the year classes could be associated with a synchronization of brood production by the most fecund females. Except for the one collection, the data show clear evidence of a greater probability of pregnancy of females with more eggs.

The relative fecundity of pregnant and nonpregnant sailfin females from Central Avenue (Table VI) is similar to that of the Rockport sample. In nine of the 13 collections with pregnant and nonpregnant ovulated females the pregnant females have a higher average number of eggs. Three of the samples show a significantly ($p < 0.01$) greater number of eggs in the pregnant females. Two of the four exceptions are late in the year when all females contain few young. The other two—June, 1960, and April, 1961, occur shortly after the start of the breeding season and may reflect to a lesser degree the same brood synchronization noted in Rockport.

Lula Sams sailfins are not presented here because sample sizes fluctuated greatly and were so low after the ditch was dredged that comparisons are impossible. They are not combined with the Central Avenue sample because interpopulation differences in fecundity might affect the results. Pregnant

TABLE VI

Average number of eggs in ovulated female sailfins collected at various times in the Central Avenue ditch. Other information is as in Table V.

Months	Not Pregnant	Partially Pregnant	Entirely Pregnant	t	n	p>
1960						
March	43.4					
April	27.7	37.9	31.3	0.45	16	0.7
May	31.6	30.5	35.8	0.82	17	0.5
June	43.6		38.6	0.54	22	0.6
July	47.9	46.0	51.8	0.58	60	0.6
August	21.1	20.0	34.6	4.33	56	0.001
September	22.9		15.6	1.88	27	0.1
October		40.0	19.4			
1961						
February	12.0					
March	48.9		85.7	3.26	22	0.01
April	75.9	63.5	75.1	0.07	45	1.0
May	64.6	94.6	80.9	3.11	76	0.01
June I	99.8	109.7	108.7	0.78	48	0.5
June II	71.0	98.9	81.6	0.58	13	0.6
July	39.0	68.7	63.4	1.96	46	0.1
August	28.9		25.3	0.63	56	0.6

females in the June II, and July, 1961, collections have significantly more eggs than nonpregnant females (probabilities of being by chance exceed 0.01 and 0.001 respectively). In none of the other samples do the probabilities exceed 0.1.

The amazon molly females from Central Avenue do not show the same pattern (Table VII). Pregnant females average more eggs in only two of the 15 collections that contain both pregnant and nonpregnant ovulated females. In these two samples the differences are far from significant. In four of the collections the increased number of eggs in the nonpregnant females has a probability of less than 0.01 of being by chance.

Pregnant amazon molly females from Lula Sams also have fewer eggs than nonpregnant ones in 12 of the 13 collections in which comparisons are possible (Table VIII). Differences in the July, 1960 sample are far from significant and may be due to chance. Three of the comparisons favoring nonpregnant females have a probability of less than 0.01 of being by chance.

These data could reflect a more efficient sperm transfer to larger (sailfin)

TABLE VII

Average number of eggs in ovulated female amazons collected at various times from the Central Avenue ditch. Other information is as in Table V.

Months	Not Pregnant	Partially Pregnant	Entirely Pregnant	t	n	p>
1960						
February	27.2	18.0				
March	29.9	14.0	28.0	0.10	290	1.0
April	33.6	22.4	8.0	2.69	155	0.01
May	33.1	26.0	28.8	1.60	252	0.2
June	55.9		49.9	0.98	168	0.4
July	77.7	94.0	69.9	1.56	250	0.2
August	52.1	23.0	55.2	0.64	211	0.6
September	39.7	53.0	32.9	1.97	185	0.1
October	37.4	41.0	28.2	1.03	48	0.4
November	100.3	35.0				
1961						
January	28.5					
February	44.4					
March	67.1	93.2	70.8	0.38	148	0.8
April	81.3	54.8	57.1	1.96	60	0.1
May	87.5	68.3	58.9	7.13	348	0.001
June I	120.9	106.9	82.9	15.58	256	0.001
June II	110.7	94.3	84.0	1.28	70	0.3
July	82.0	66.8	44.6	2.44	152	0.02
August	61.9	52.6	43.9	3.04	270	0.01

TABLE VIII

Average number of eggs in ovulated female amazons collected at various times from Lula Sams. Other information is as in Table V.

Months	Not Pregnant	Partially Pregnant	Entirely Pregnant	t	n	p>
1960						
February	17.4					
March	45.3					
April	31.0					
May	41.1	32.7	26.0	2.99	73	0.01
June	28.9	21.4	22.8	3.66	229	0.001
July	36.7	39.0	37.0	0.08	29	1.0
August	67.4	53.4	56.1	0.98	44	0.4
September	60.5	51.4	21.8	2.00	67	0.05
October	38.9	38.8	27.8	0.64	31	0.6
November	29.4	4.0	20.0	0.30	11	0.8
December	33.0					
1961						
January	17.5					
February	39.3					
March	74.0	62.0				
April	65.8	30.5	30.8	2.43	144	0.02
May	61.7	49.3	33.3	6.31	221	0.001
June I	71.7	66.2	57.8	2.57	192	0.02
June II	58.5	54.3	52.4	2.31	378	0.05
July	61.5	50.4	58.8	0.40	196	0.7
August	69.9	77.5	42.0	1.01	376	0.4
September	43.9	83.0				

or smaller (amazon) females, or "preference" for fatter (sailfin) or thinner (amazon) females. I believe that both occur. It is probable that the males are discriminating; however, it is also possible that differences in the female's response to courting males is involved.

Relationship of Pregnancy and Female Length

The number of eggs produced by a given female fish is in large part dependent upon her size in that the number of eggs tends to increase with the female's length. If there is a difference in the relative reproductive efficiency of various sized females, the rate of recruitment might be affected. The pregnancy percentages of molly females reflect their reproductive efficiency; therefore, it is possible to compare the frequency of pregnancy in different sized females belonging to each of the two species and obtain an evaluation of the relative reproductive efficiency.

The pregnancy percentages for the ovulated females obtained during the middle of the reproductive season in 1960 (April through November) are presented in Fig. 12. Samples obtained earlier in the spring are excluded because in some, most females had ovulated but were not pregnant. The fact that the smaller amazon mollies were often not pregnant, was probably due to infrequent overall brood production. That is, sailfin males so infrequently copulated with amazon females that young females remain nonpregnant for some time after ovulation. The pregnancy percentage was rather high in those females measuring between 40 and 55 mm., then it steadily declined in the longer size classes. A slight rise was indicated in the 70 to 75 mm. sample from Central Avenue, but was based on a single female and was probably not significant. The pregnancy percentages of amazon females from Central Avenue were usually lower than those of comparable size from Lula Sams which reflected the lower Central Avenue pregnancy ratio shown in Fig. 10. The gradual decrease of pregnancy frequency with size was best shown in the Central Avenue sample, probably as a result of the large sample obtained there.

Comparable pregnancy percentages for sailfin females were much higher, fluctuate widely, and did not decline with size. The only indication of a decrease was in the sailfin sample from Lula Sams. The percentages were over 40 per cent up to 60 mm., after which the sample size was so small that the data were inconclusive. The Central Avenue figures indicated little or no size preference. The largest numbers of female sailfins were obtained at Rockport and these pregnancy percentages most clearly showed an increase with size.

A similar pattern occurred in 1961. The same gradual decline in pregnancy with size occurred in the amazon females (Fig. 13). In this year the Lula

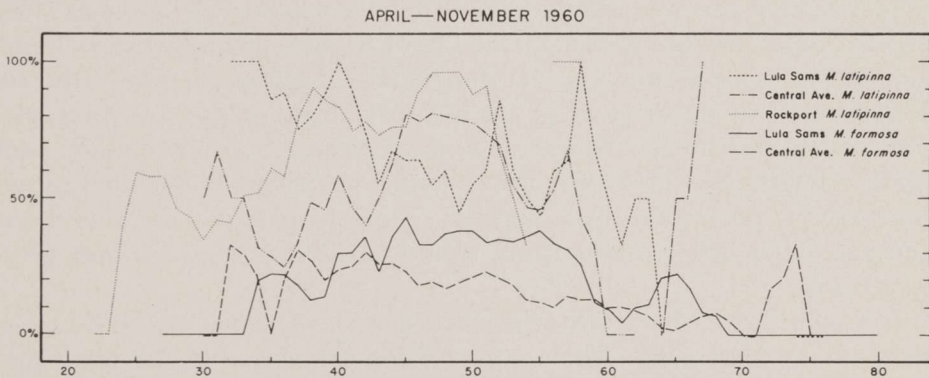


FIG. 12. Percentage of the ovulated females of various sizes that were pregnant between April and November, 1960. Three point rounded curves were used to reduce any possible recording preference for or against any millimeter reading.

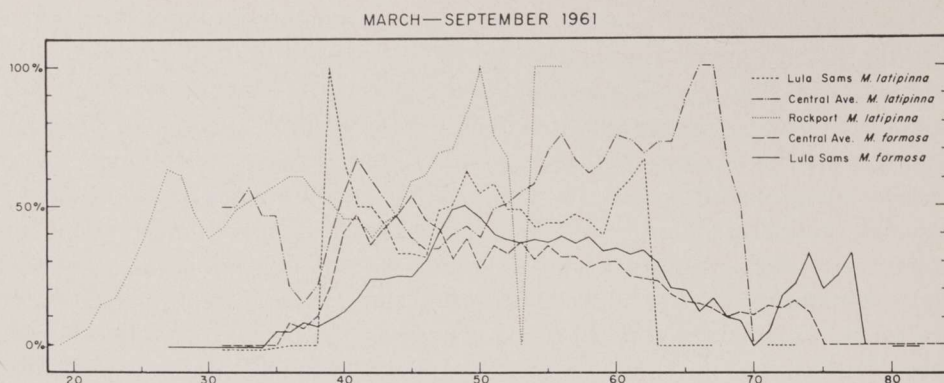


FIG. 13. Percentage of the ovulated females of various sizes that were pregnant between March and September, 1961. Other information is as described for Fig. 12.

Sams amazon females above 70 mm. showed a slight pregnancy increase. However, this was based on so few fish that sample size was a likely factor. The differences between the two populations were much less pronounced than in 1960 which may have reflected the increased number of male sailfins present in 1961 at Central Avenue.

The percentages for Lula Sams sailfin females were high in the middle-size classes and low on each end, which may well have been caused by the small sample available. Both the Central Avenue and Rockport samples showed a gradual increase in pregnancy with size. The decreases at 53 mm. at Rockport and 70 mm. at Central Avenue were based on single fish. The figures at 50 mm. and 67 mm. respectively were derived from much larger samples.

The size differences in pregnancy percentages may be affected by seasonal changes in overall pregnancy rates associated with growth of a prominent size class. If few females were pregnant at the time most females were a given size, that size group might appear to be less likely to be pregnant than slightly longer females obtained when most females were pregnant. Therefore, I have plotted the data by individual collections to show that the differences between the species are real. Moreover, analysis of pregnancy with size by individual collections may show some details obscured by pooling the samples. Because of the small numbers of sailfins obtained in Brownsville, I have pooled the two localities. Separate plots do not reveal any interpopulation differentiation that would be obscured by combining the populations.

The sailfins from Rockport are shown in Fig. 14. None of the fish were pregnant in April, 1960. There was a distinct increase in the pregnancy percentage with size in the May collection. The effects of sexual preference should be most noticeable at the start of the breeding season because sperm retention would have the least distorting effect. The June collection was the reciprocal of the May collection. These data are similar to those shown in

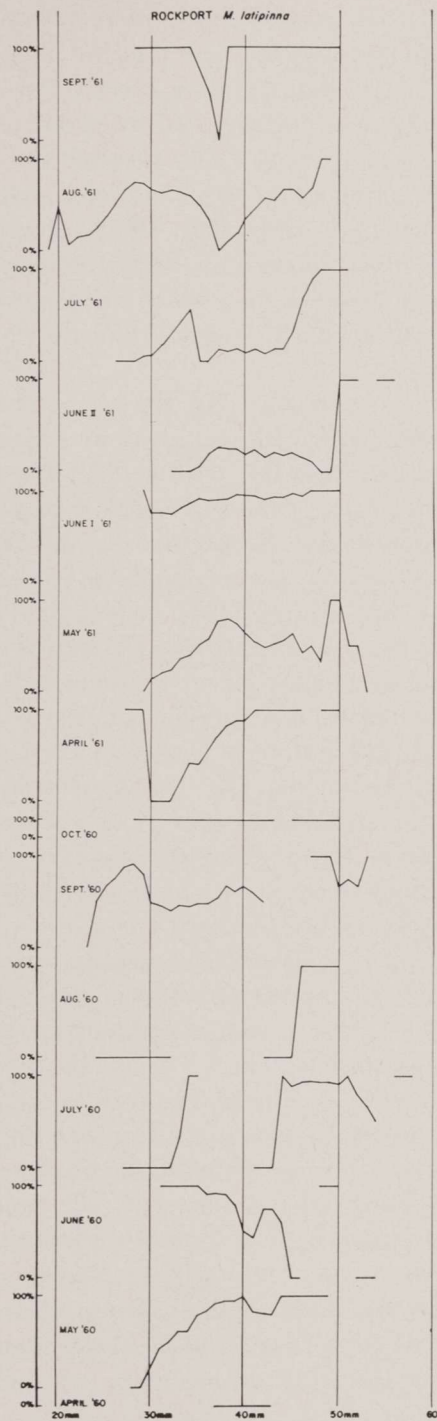


FIG. 14. Pregnancy percentages of the ovulated sailfin females from near Rockport by month. Other information is as described for Fig. 12.

Table V. I believe that the smaller fish, which were not pregnant in May, had subsequently been impregnated, and that the larger fish, which were all pregnant in May, had just produced their broods and had eggs which were in early developmental stages and were recorded as being without embryos. The newly-born young could pass easily through the nets in June and be collected first in July as noted in Fig. 7. There were two size classes of ovulated females in the July collection: small, nonpregnant females and large, pregnant ones. Only the largest females collected in August were pregnant. The September collection which contained several small, nonpregnant fish had most females pregnant. All of the females in the October collection were pregnant.

The 1961 collections are similar, but the changes are less pronounced. No collections were obtained which contained exclusively nonpregnant ovulated females. The larger fish in the April collection were more likely to be pregnant than the smaller ones. The early peak is based on one fish and the following valley is based on five. These six fish were 16.7 per cent pregnant and if this figure was used the curve would be very similar to that of the May, 1960 collection. In the May, 1961 collection there were few small, pregnant females and many of the 40–50 mm. fish were pregnant. The 100 per cent and 0 per cent pregnancy percentages of the largest fish were based on one fish each. Most females were pregnant in the June I, 1961, collection; however, the few that were not were mostly small. The 100 per cent pregnancy at 29 mm. was based on one fish. The increase in pregnancy with size in the June II and July collections was more apparent than real as few large fish were obtained. In the August collection, pregnancy frequency increased with size. Because most of the fish in the September collection were pregnant, no trends can be ascertained.

The curves obtained from sailfins collected in Brownsville are more suspect because the much smaller samples necessitated the pooling of data obtained from two stations. None of the females were pregnant in March, 1960 (Fig. 15). Most females were pregnant in April, but the smallest ones were not. The May collection tended to reverse the April trend. These results can be explained by assuming that the larger females had just produced broods and the next group of eggs were in such early developmental stages that they were tabulated as ovulated but not pregnant. The June and August collections fluctuated widely with larger and smaller fish least often pregnant. The July collection was the reverse with all the largest and smallest females pregnant; however, the highest incidence of nonpregnant females was among the smaller fish. The six large females in the September collection were not pregnant and many of the smaller ones were. All females in the October collection were pregnant.

Similar inconclusive results are apparent in the 1961 sailfin samples from

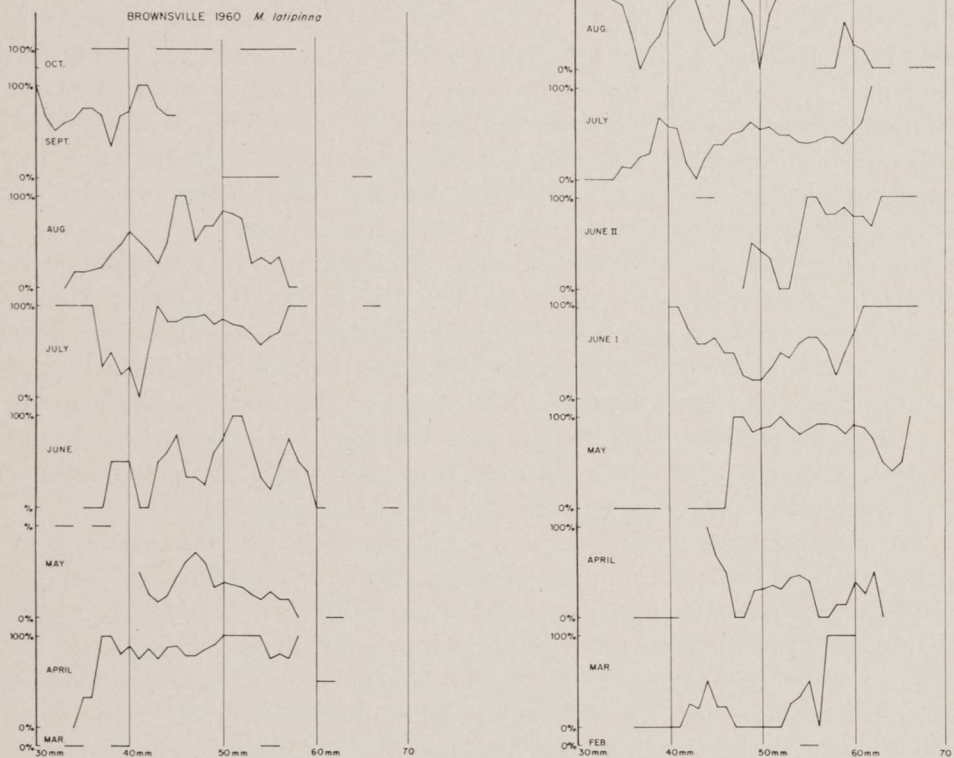


FIG. 15. Pregnancy percentages of the ovulated sailfin females from Brownsville by month. Other information is as described for Fig. 12.

Brownsville. The single ovulated female was not pregnant in February. A few of the females collected in March were pregnant and most of them were large. The April collection fluctuated widely. Most of the females in the May collection were pregnant and many of those that were not were small. The June I collection was inconclusive as the large and small females were more often pregnant than the middle sized ones; however, the 100 per cent pregnancies were based on one small and 16 large females. Most females in the June II collection were pregnant and the low figures were for the small females. The pregnancy percentages for large females collected in July was much higher than that for small females. This was the largest sample available. The August and September collections fluctuated widely.

The data obtained from amazon females from Central Avenue were differ-

ent from those based on sailfin females (Fig. 16). The one pregnant female in February, 1960 and two in March were smaller than average. Small females were more often pregnant in April and May. The data for June are less conclusive, but pregnancy becomes less frequent with size in females over 50 mm. as it does in the July sample. The August collection data are inconclusive, perhaps because of the 1960 year class females entered the breeding population, and these fish would have had less time to become pregnant. Nevertheless, there was a decrease in pregnancy of females over 50 mm. Pregnancy was inversely correlated with size in females over 45 mm. collected in September. The October collection may be the same but the small sample size made any conclusion tentative. The November collection contained four ovulated females, one of which was pregnant.

Similar figures are available for 1961. None of the females obtained in January and February were pregnant. The data for fish collected in March, April, and May are inconclusive. The two collections obtained in June show a larger pregnancy percentage for small females than for larger ones. Except for the 1961 year class (females under 45 mm.), the July collection shows the same pattern. Obviously the younger females have scarcely had time to become pregnant. The data obtained from the August collection clearly shows a decline in pregnancy percentage with increasing size.

The data obtained for amazon females from Lula Sams followed the same pattern as that for amazon females from Central Avenue (Fig. 17). None of the females obtained in February and March, 1960 were pregnant. The April data were inconclusive as the high pregnancy percentage at 58 mm. and the absence of pregnancy around 61, 70, and 80 mm. were all based on samples of one or two females. The May figures were also questionable but more of the pregnant females were small. The June, July, and August data all showed a trend for reduced incidence of pregnancy with size. The September and October data were less conclusive, in part because the smallest fish (1960 year class) were often not pregnant and the November data were inconclusive because of sample size. None of the December fish were pregnant.

The 1961 figures are similar. None of the females collected in January and February were pregnant, one female was pregnant in March, and in the April and May samples the smaller females were more often pregnant. The rise in percentage at 68 mm. in May was based on one pregnant female and the adjacent 0 per cent pregnancies were based on a total of 10 females. The two June collections also showed a decrease in pregnancy percentage with increased size. The increased percentage of pregnancy in the 73 mm. class in the June I collection is also based on one female. Ovulating young of the year had not had time to be inseminated by the time of the June II collection. The July and August collections were inconclusive, in part because of the young of the year; however, both showed a decrease in pregnancy with size over 50 mm. The rise at 72 mm. in July was based on a

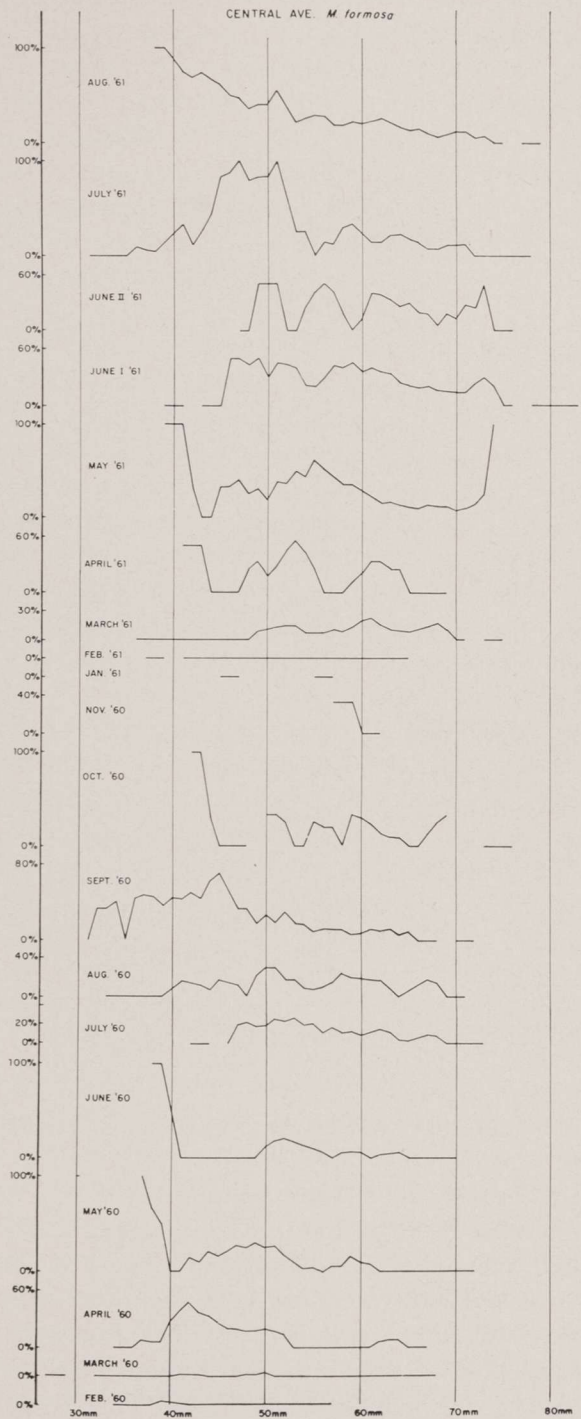


FIG. 16. Pregnancy percentages of the ovulated amazon females from Central Avenue by month. Other information is as described in Fig. 12.

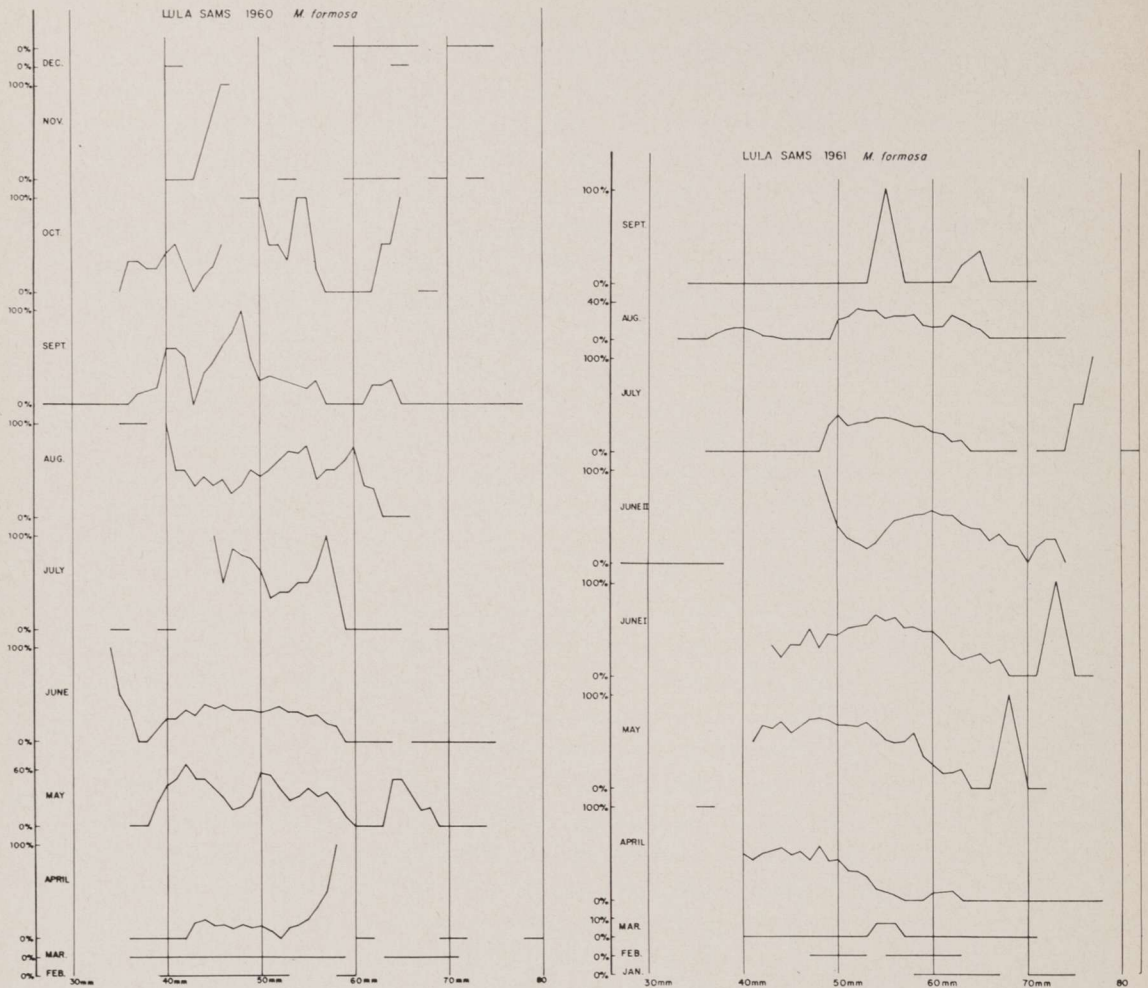


FIG. 17. Pregnancy percentages of the ovulated amazon females from Lula Sams by month. Other information is as described in Fig. 12.

single fish, and the September data were probably distorted by the effects of Hurricane Carla.

Many of the figures showing a relationship of size with pregnancy are less conclusive than desired; however the trends for sailfins differ from those for amazons. Pregnant females were often present in the first collections of sailfins, which included ovulated young of the year (June, 1960, and July, 1961, in Rockport; July, 1960 and 1961 in Brownsville), whereas the young amazons are seldom pregnant so early (July and August, 1960, and July, 1961, at Central Avenue; July and September, 1960, and June II, July and August, 1961, at Lula Sams). This is thought to reflect a high frequency of copula-

tion with sailfin females, causing pregnancy to follow ovulation closely, and a corresponding low frequency of copulation with amazon females, causing them to remain nonproductive for some time after ovulation. The smallest fish would seldom be pregnant and would have the fewest eggs, which should reduce the average number of eggs in nonpregnant females. Because small amazons remain nonpregnant longer than do small sailfins the amazons would have a greater tendency to have fewer eggs in nonpregnant than in pregnant females. As the data presented in Tables V-VIII show the reverse, selection favoring more fecund sailfins and less fecund amazons must be strong.

Secondarily, the largest amazon females tend not to be pregnant and the largest sailfin females are at least no less likely to be pregnant than smaller females. This is thought to reflect a reduction of copulation with large amazon females that does not occur in sailfins. On the contrary, there is some inconclusive evidence that copulation is most common with larger sailfin females. Amazon females are longer than sailfin females so that the largest amazons might be selected against because they are too long; however, reduction in pregnancy percentage is apparent at about 50 mm., well within the size range of sailfin females. The presence of large pregnant amazons may result from storage of sperm from a time when the female was smaller and does not necessarily indicate that males copulate with these large females. In the 1960 collections from Central Avenue there were more pregnant amazons in the 43 mm. size class in April, 50 mm. in May, and 52 mm. in June. Pregnancy in the 52 mm. fish in June may have resulted from copulation when these females were 43 mm. long in April. Thirdly, the pregnancy percentage for any given size class of sailfins is higher than that for equal-sized amazons. Obviously, there is an overall higher pregnancy frequency in sailfins.

Relationship of Pregnancy and Fecundity of Equal-sized Females

Although large females tend to produce more eggs at any given time than do small females, considerable variation exists in each size class. Any difference in the pregnancy frequency of these more and less fecund females would increase or reduce the recruitment of young.

Analysis of the average number of eggs in nonpregnant, partially pregnant, and entirely pregnant female sailfins from Rockport is given in Fig. 18. The data for April, May, and June, 1960, do not indicate preference within any size class. In July pregnant females had many more eggs than did their nonpregnant counterparts. The same is indicated but not proven for August because of the absence of overlapping sizes. In September the nonpregnant females again had fewer eggs than the pregnant ones. No conclusions can be

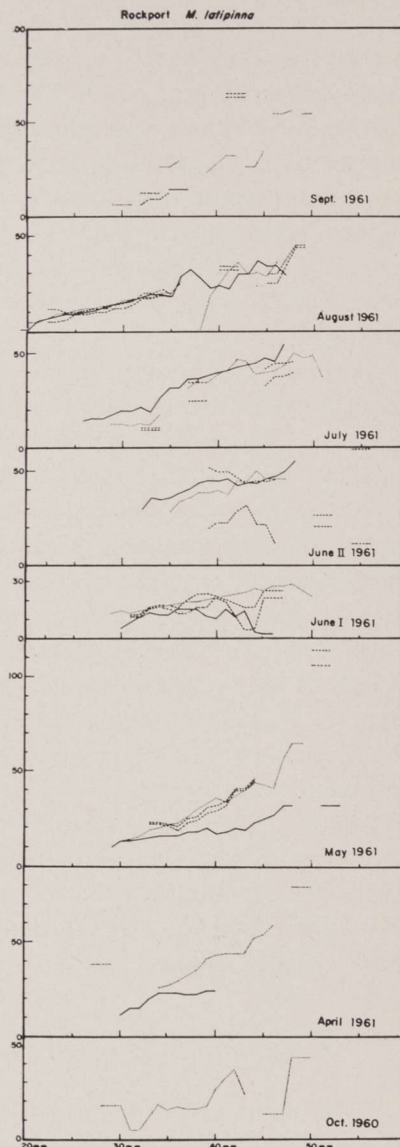
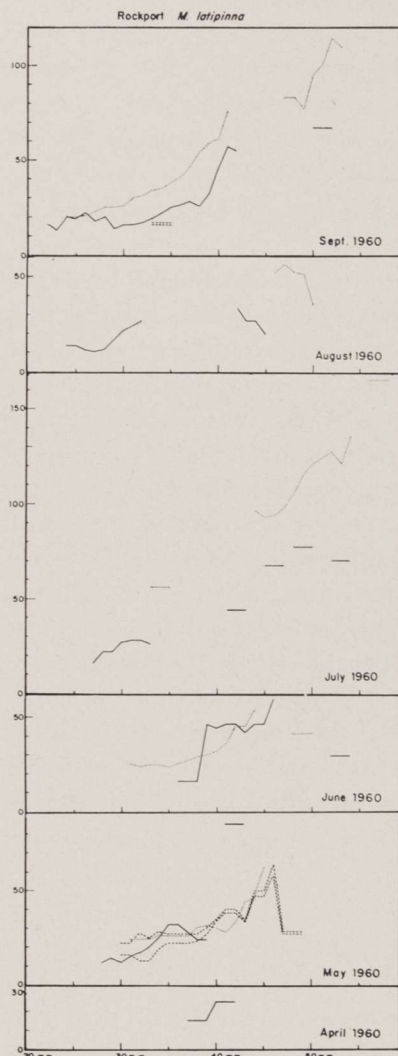


FIG. 18. Average number (abscissa) of eggs in nonpregnant (solid line), partially pregnant (dashed line) and entirely pregnant (dotted line) sailfin females of various sizes collected near Rockport. Two lines are presented for the partially pregnant females: the lower represents the average number of fertilized eggs, and the upper shows the average number of the eggs with and without embryos. The average number of eggs without embryos can be ascertained by determining the distance between the lines. Three point rounded curves were used to reduce the possible recording preference for or against any single millimeter reading.

derived from the October data because all ovulated females collected were pregnant. Pregnant females average more eggs than do equal-sized nonpregnant ones in April, May, and June I, 1961. In the last two collections there are reasonably adequate samples of partially pregnant females in which the total egg complements average higher than do the complements of the nonpregnant females and approximate those of fully pregnant females. Surprisingly, the average number of embryos in partially pregnant females in May always exceeded the total complement of nonpregnant females in the same collection. Calculations for June II, July, and August are inconclusive. The single nonpregnant female in September had fewer eggs than equal-sized pregnant females; however, this could be due to chance. In five of the collections the average number of eggs in pregnant females is consistently above the number in nonpregnant ones, and none of the collections show the reverse.

The plots for sailfins from Central Avenue show the same preference for more fecund females (Fig. 19). All ovulated females in March, 1960, are nonpregnant females and approximate those of fully pregnant females ever, the nonpregnant ones are less fecund in the 40 to 42 mm. size group where the sample size is most adequate. Pregnant females averaged more eggs than equal-sized nonpregnant ones in May. The data for June and July overlap considerably. In the August collection the pregnant females again contain a higher average number of eggs. The same occurred in the September collection, but only small females were pregnant. All females were pregnant in October. The data for 1961 are less conclusive, but the trend remains. The single ovulated female collected in February was not pregnant. The March data are not conclusive, but the pregnant females between 41 and 43 mm., where the sample size is greatest, have more eggs than nonpregnant ones of equal size. The April collection is inconclusive, but the May collection clearly shows more eggs in pregnant females, especially between 54 and 63 mm., where the sample size is most adequate. The subsequent collections are again inconclusive. In four of the collections pregnant females contained more eggs than nonpregnant females of the same size and none show the opposite; therefore, the most fecund sailfin females of a given size are most likely to be pregnant.

Central Avenue and Lula Sams sailfins are not combined because interpopulation differences in relative fecundity would distort the data. Data from Lula Sams sailfins are not plotted here because the sample sizes are often so small that the data are of little meaning; however, in none of the collections do nonpregnant females have more eggs than equal-sized pregnant females. The June II and July, 1961, collections show a significantly greater number of eggs in pregnant females than in their equal-sized nonpregnant counterparts.

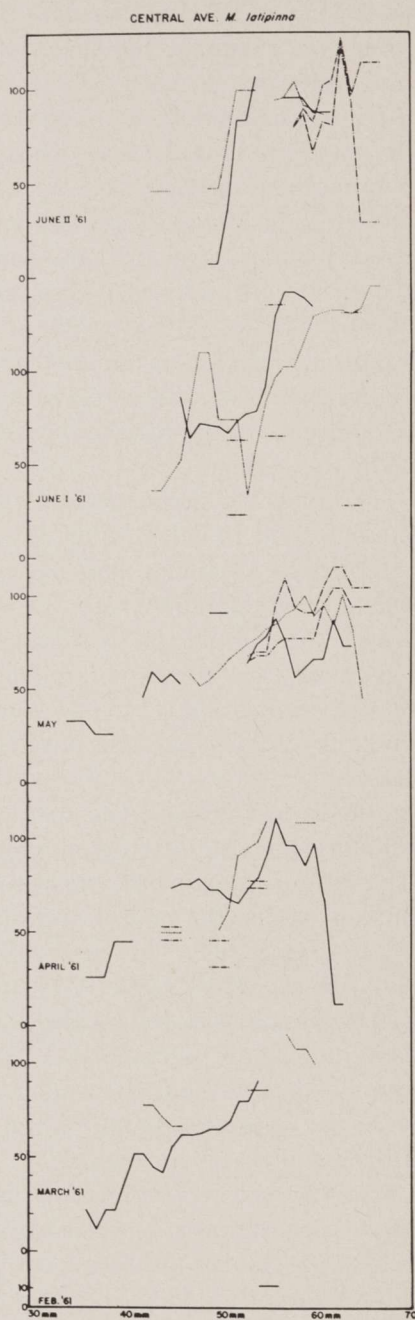
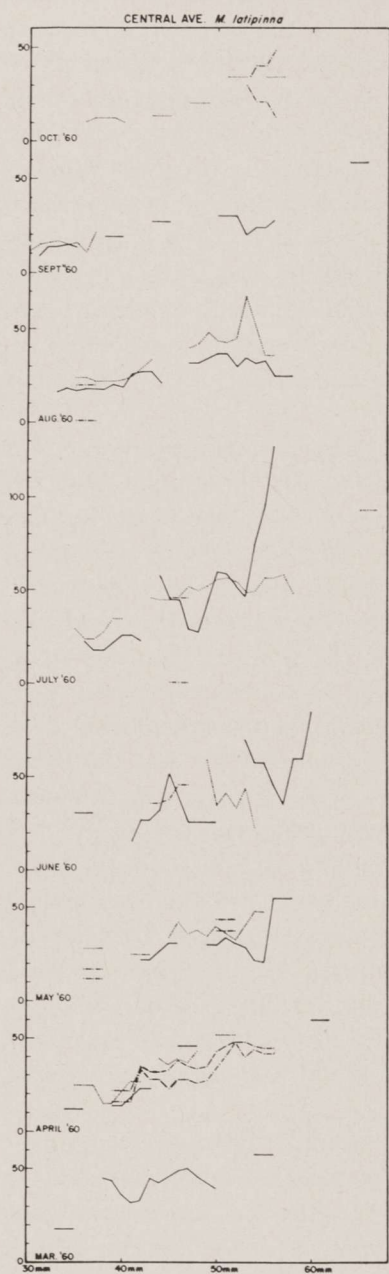


FIG. 19 (continued, p. 53).

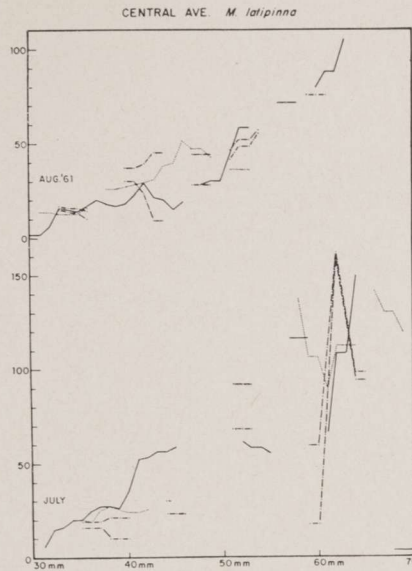


FIG. 19. Average number of eggs in nonpregnant, partially pregnant, and entirely pregnant sailfin females of various sizes collected at Central Avenue. Other information is as described for Fig. 18.

Somewhat different results occur when the Central Avenue amazons are plotted in the same manner (Fig. 20). Nonpregnant females averaged more eggs than the pregnant or partially pregnant females of the same size in March and April, although the sample size is too small for a definite conclusion in March. The data for the rest of 1960 is inconclusive. No females were pregnant in January and February, 1961, and the data are again inconclusive in March and April. Nonpregnant females averaged more eggs than the variously pregnant ones in May, and the two June, 1961 collections. There is some overlap but the pattern is obvious. The July and August collections are again inconclusive. All four of the collections in which the number of eggs is different in pregnant and nonpregnant females indicate that nonpregnant females have the most eggs.

The amazon females from Lula Sams show the same pattern as those from Central Avenue (Fig. 21). None of the ovulated fish were pregnant in February and March, 1960. The April data are suggestive in that in nine of the 13 size groups with comparable data, the nonpregnant females have more eggs than do the pregnant ones. The May and especially the June data definitely favor the hypothesis of more eggs in nonpregnant females. The July data are inconclusive, and in August the nonpregnant females usually had more eggs than either kind of pregnant females. September, October, and November data are inconclusive, and the December, 1960, January and February, 1961, collections contained no pregnant females. The partially pregnant female in

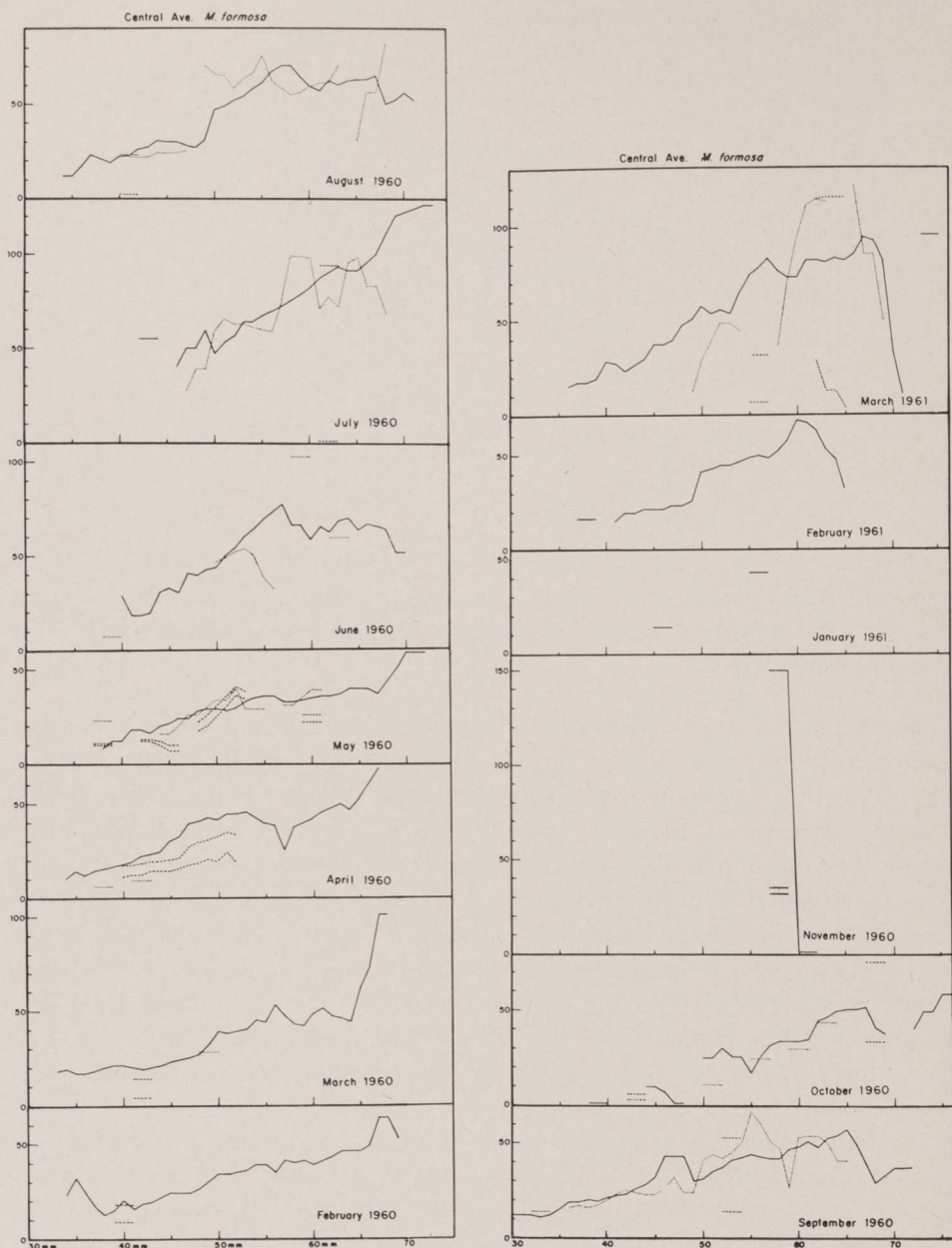


FIG. 20 (continued, p. 55).

March had fewer eggs than the average for nonpregnant females of the same size. Nonpregnant females in the April collection consistently had more eggs than the partially pregnant ones; however the fully pregnant females fluctuated so much that a similar conclusion could not be drawn. Although the differences were minimal, there was a higher average number

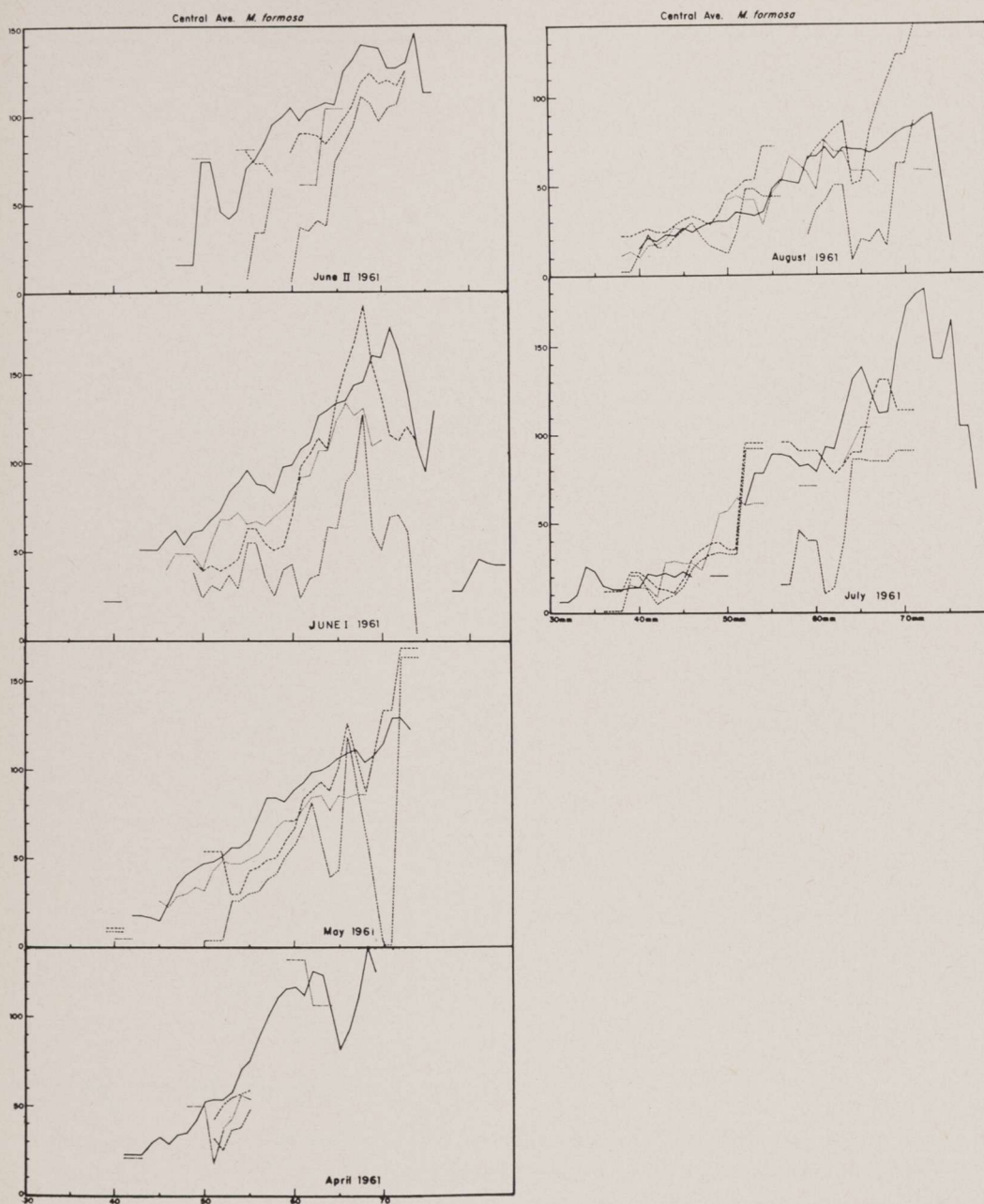


FIG. 20. Average number of eggs in nonpregnant, partially pregnant, and entirely pregnant amazon females of various sizes collected at Central Avenue. Other information is as described for Fig. 18.

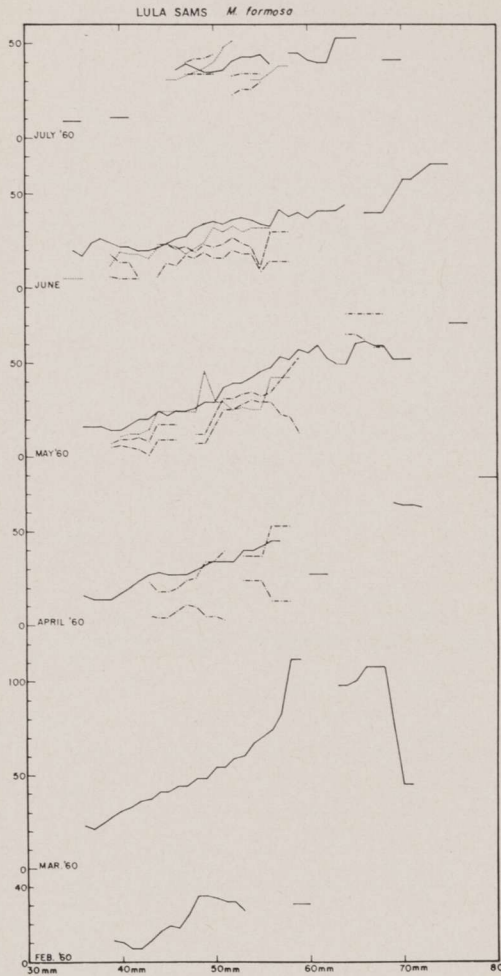
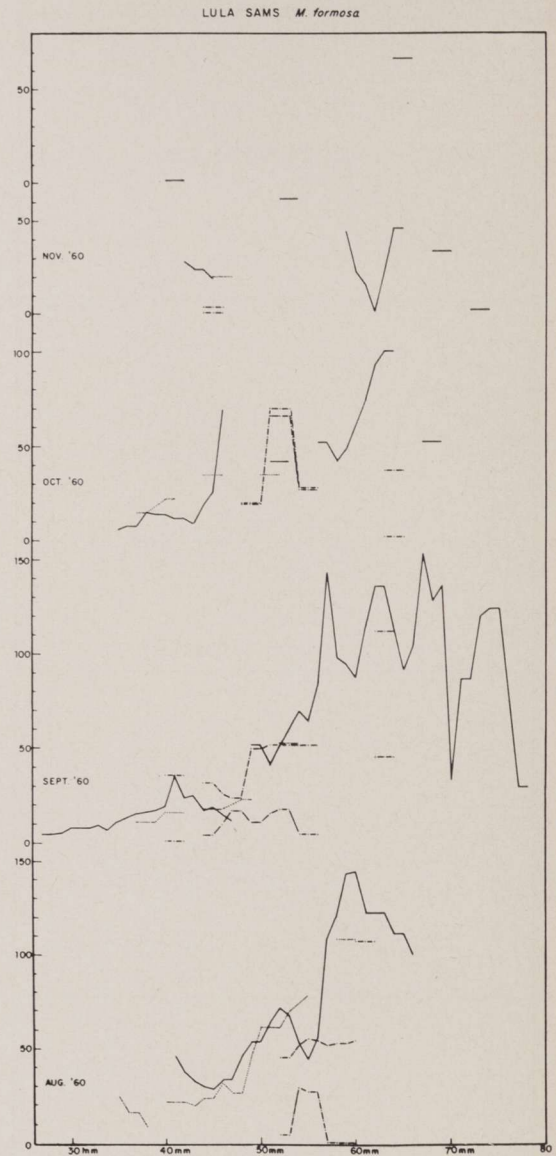


FIG. 21 (continued, p. 57).



of eggs in nonpregnant females collected in May than in either category of pregnant females. The June I collection is inconclusive. The June II collection is similar to the April collection in that the partially pregnant females averaged fewer eggs than the nonpregnant ones, but the pregnant ones varied so much that any conclusion would be questionable. The July, August, and September collections are inconclusive. In all conclusions in which there is a difference the nonpregnant females have the highest number of eggs.

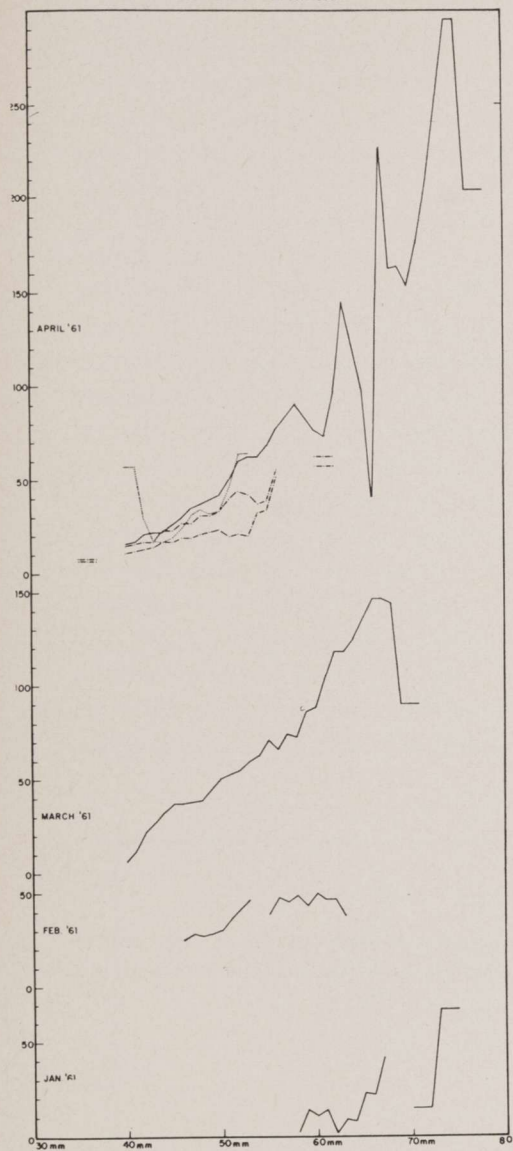
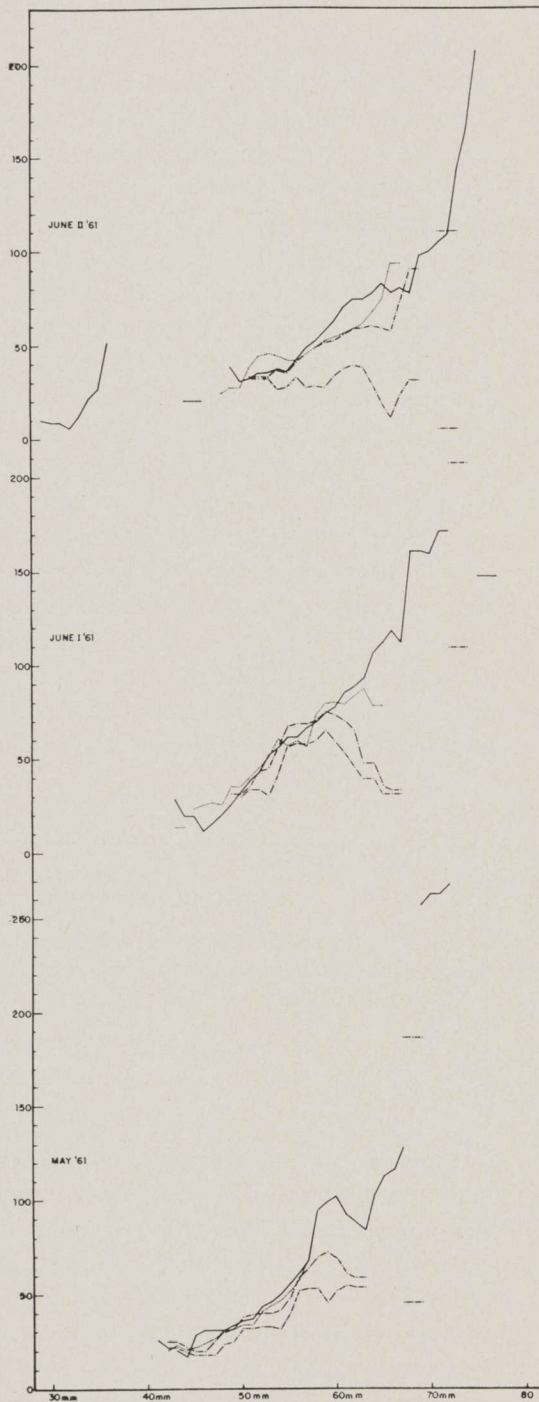


FIG. 21 (continued, p. 58).



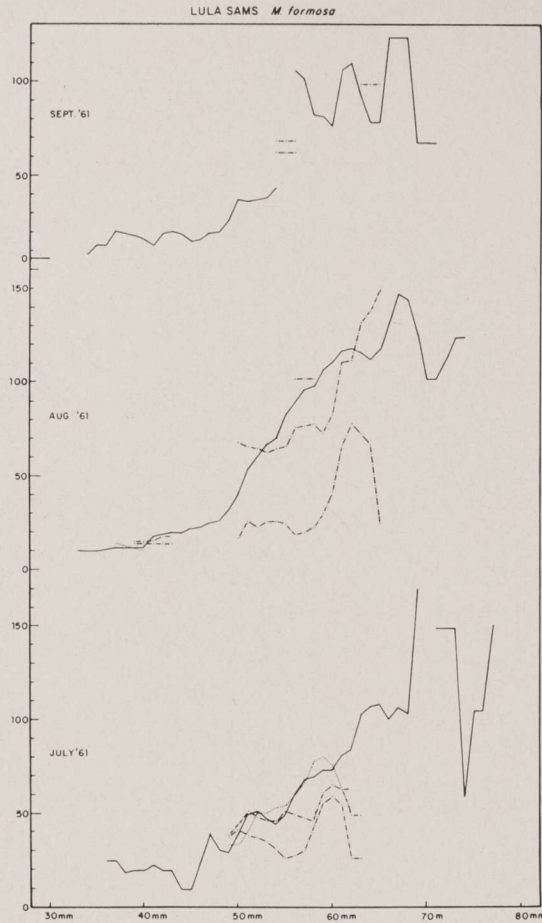


FIG. 21. Average number of eggs in nonpregnant, partially pregnant, and entirely pregnant amazon females of various sizes collected at Lula Sams. Other information is as described for Fig. 18.

Many collection samples show no differences in fecundity between equal-sized pregnant and nonpregnant ovulated mollies. Without exception, where differences occur, the sailfin samples show a consistently greater fecundity in pregnant females and the amazon samples indicate a consistently greater fecundity in nonpregnant females. There must be a greater differential in preference by the males than is shown by these figures, because some of the analyzed females may have retained sperm from a previous courtship at which time they may have had different relative fecundities. Such females would tend to obscure the differences.

Percentage of Eggs with Embryos

The relative efficiency of the reproductive activity of a given population can be estimated by ascertaining the fraction of mature eggs that develop. Subsequent reductions in this number reflect other environmental factors such as predation, thermal extremes, etc. Studies on viviparous animals can include analyses of relative reproductive efficiency because the females retain the eggs for some time after development and their developmental history can be traced. Comparison of the number of mature eggs with and without embryos provides a rather reliable estimate of reproductive efficiency of the various populations studied (Fig. 22). These figures are a slightly more accurate representation of the recruitment advantage of sailfin females than those shown in Fig. 11, in which relative numbers of pregnant females were contrasted. Moreover, use of individual eggs increases the sample size sufficiently so that the two sailfin populations in Brownsville can be plotted separately. Eggs in sailfin females collected in Brownsville are far more likely to have embryos than are eggs in amazon females from the same collection, and the functional reproductive season of amazons starts later than does sailfin reproduction, despite the fact that the amazons ovulate first. Each yearly sample of sailfin adults from Brownsville indicates a reproductive peak in March or April, whereas the corresponding first peaks in amazons are May or later. The numerous young sailfins noted in early season collections substantiate the differential reproduction. Comparisons early in

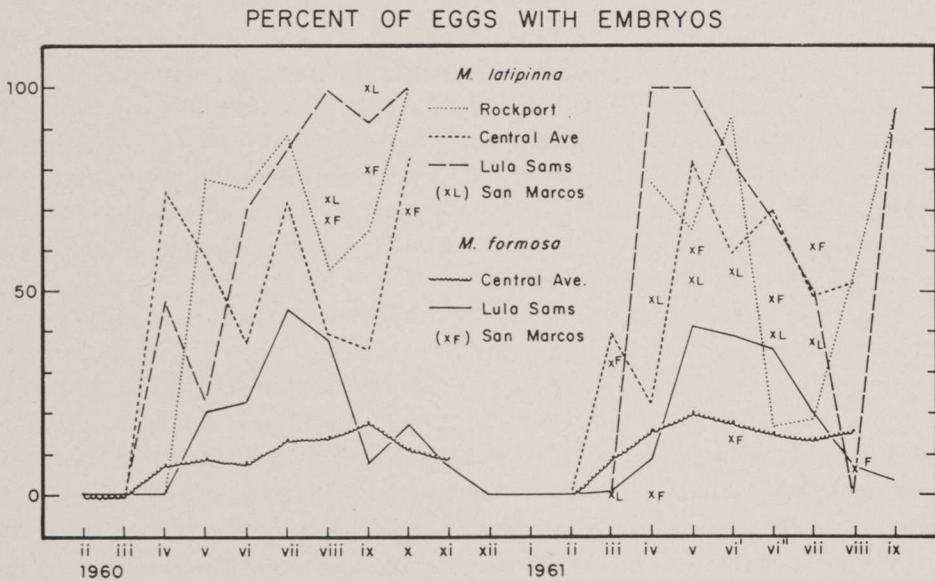


FIG. 22. Percentage of the eggs with embryos. The females from each collection are plotted separately.

the reproductive season are not likely to be distorted by sperm retention. The resulting young might have a relatively high survival since they would not have to compete with numerous older individuals of their year class. Comparison of the relative reproductive efficiency of sailfins and amazons at Central Avenue and Lula Sams in 1960 may explain the change in population density at Central Avenue. In 1960 approximately 50 per cent of the Central Avenue sailfin eggs and 10 per cent of the Central Avenue amazon eggs had embryos. At the same time approximately 70 per cent of the Lula Sams sailfin eggs and 25 per cent of the Lula Sams amazon eggs had embryos. This relationship should result in the relative increase in sailfin population density that occurred at Central Avenue.

The fecundity relationships in amazon populations remains the same as those discussed with reference to the pregnancy percentages: *i.e.* Lula Sams had higher percentages than Central Avenue and Central Avenue had higher percentages in 1961 than in 1960. This probably reflects a positive association with the population density of sailfin males. Sailfin pregnancies do not appear to respond to the same factors in such a pronounced manner.

These data are all in accord with a hypothesis that sailfin males would first court and copulate with sailfin females in the males' territory. The first sailfin females courted would be the most fecund, and subsequent courtship would be with females of decreasing fecundity. After most sailfin females are pregnant, the males would court and copulate with the amazon females in the territory. The first amazons courted would be the least fecund and subsequent courtship would be with females of increasing fecundity. Most male fish are able to father young by several females; therefore, if amazons are scarce, most would be fertilized. Increases in the amazon population density above a "critical point" would in effect result in some amazons being excluded from the breeding population. Under these conditions of high density of amazons, those with the greatest reproductive potential would not contribute to brood production. A similar "critical point" must exist in the sailfin sex ratio, but in this instance the superfluous females would include those with the least reproductive potential.

Males are territorial against other males. The females swim about and are courted as they pass through the various territories. The pattern is similar to that shown for *Cyprinodon* (Raney, *et al.*, 1953; Barlow, 1960). Although Raney, *et al.* (1953) did not report this phenomenon in *Mollienesia latipinna* from Florida, it is possible that they overlooked it as male mollies have far larger territories than male *Cyprinodon*, with which they were primarily concerned. Because the population density would vary between territories, the pooling of results from several territories would obscure the precision of the discrimination in any given territory.

The large number of apparently unfertilized eggs of *Ambystoma jeffer-*

sonianum laid in areas known to be occupied by large numbers of the all-female triploid (Uzzell, 1962) may involve similar differences in courtship interactions. Unfortunately, the source of these unfertilized eggs is unknown and will be difficult to ascertain in these oviparous animals. However, their absence in areas outside the all-female range leads me to suspect that the all-female salamanders are subjected to a reproductive check similar to that shown for amazon mollies.

Figure 22 also presents the percentage of the San Marcos sailfin and amazon molly eggs that contain embryos. The figures for the two species are nearly equal. A drastic increase of amazon mollies and a corresponding decrease of sailfins occurred during the same interval. Amazon mollies were introduced into a flourishing sailfin population in San Marcos in 1955. They were obtained in small numbers in 1958, and by 1960 amazon populations approximated the size of sailfin populations. In the spring of 1962 sailfins were about 10 per cent as common as the amazons, and most of the sailfins were found at collection sites distant from the amazon molly release point. It is possible that these data reflect the correlation of sailfin density and amazon pregnancy noted at Brownsville. It is also possible that the sailfin males at San Marcos are less discriminating since they probably descended from allopatric Louisiana stocks (Brown, 1953). The low discrimination by allopatric males from Austin leads me to favor the second explanation. Furthermore, a comparison of the number of eggs in pregnant and nonpregnant female amazons from San Marcos shows very different results than those shown for Brownsville amazons in Tables VII and VIII. In four of the six collections that had comparable data, the pregnant females had more eggs. The probability of significance of those four slightly exceeded 0.001, 0.02, 0.05, and 0.1 whereas the probabilities in the two others were less than 0.5 and 1.0. Similarly, in a San Marcos live sample one-fourth of the sailfin females and one-third of the amazons' produced broods. This was the only live sample in which a larger fraction of the amazon females produced young. This information, together with the sex ratio data strongly supports the concept of strong selection in sympatric stocks of sailfins to reduce the reproductive efficiency of amazons and the absence of such selection in allopatric stocks.

Seasonal Variations in Reproductive Potential of Females in a Single Population

The data presented in Figs. 18-21 pertain to some reproductive phenomena less directly concerned with the interreaction between sailfin and amazon mollies. Virtually all of the averages show that intermediate sized females have more eggs than smaller ones. Many of the increases appear to be linear; others, including Central Avenue amazons from March 1960, February and July, 1961, and those from Lula Sams between April and June,

1961, better fit a geometric increase. Obviously a geometric increase results in far more young produced by large females and a general overall increase in recruitment.

Another major intrapopulation variation is the monthly difference in the average number of eggs in equal-sized females. Females from mid-summer collections usually average more eggs than do equal-sized females from spring and fall collections. This is very apparent in the 1960 Rockport collections (see also Tables V-VIII). Occasional summer collections such as the August, 1960 Rockport sample also average few eggs. The number of fish obtained in these collections appears to be high and the individual fish appear to be undernourished. Krumholz (1948) noted a seasonal decline in brood size in Illinois *Gambusia*, a phenomenon which he attributed to aging. My data on Texas *Mollienesia* cover a longer interval of increasing daylight which appears to coincide with the increasing brood size. Decrease in brood size in Illinois *Gambusia* and Texas *Mollienesia* correlate with decreasing daylight.

A third intrapopulation variation involves the decrease in number of eggs produced by the largest females in contrast to the numbers produced by middle-sized females. I would call this phenomenon reproductive senescence. This decrease is apparent in the Central Avenue amazon females collected in June and August, 1960 and February, March, June I, and July, 1961. The absence or presence of fully efficient reproduction among the potentially most productive females would have an important effect upon the relative numbers of young produced by the population. Moreover, the size at which reproductive senescence sets in varies from month to month (*i.e.* apparently at 60 mm. in February and 70 mm. in June I and July, 1961). Krumholz (1948) also noted this phenomenon in Illinois *Gambusia* and pointed out that the largest and oldest females were often effectively agonadal, a condition he called reproductive senility. Similarly, the largest mollies in my samples were often not ovulated. For instance, in May, 1960, when 98 per cent of the females had ovulated, no eggs were noted in three of four females over 71 mm. These females resembled Krumholz' largest *Gambusia* females in that the gonad was almost entirely absent.

Reproductive potential results from the interaction of genetic and environmental factors. Differences between the reproductive potential of samples obtained at different times from the same locality strongly support the significance of environmental effect on a genetic limit. Although a gradual seasonal change may be attributed to genetic causes, sudden changes in sequential collections such as the July (high), August (low), and September (high), 1960, samples of Rockport sailfin females must involve environmental factors. The occurrence of this phenomenon in amazon mollies is pertinent since Kallman (1962 b) has shown that there is little genetic variation in natural popu-

lations. Among the factors that are involved are available food supply and basal metabolism. As *Mollienesia* feeds extensively on a primary food source, periphyton, food production would closely follow light duration. The increase in brood size in the spring and the decrease in the fall seems to reflect seasonal changes in sunlight. Reproductive responses would lag behind the food supply because stored food could prolong a favorable interval, and tissues might need repair after an unfavorable time. Moreover, ovulation precedes brood production by several weeks. The general pattern could be modified by other factors such as long periods of cloudy weather, environmental extremes (floods, etc.), and changes in the number of individuals utilizing the available food. For instance, reproductive potential was low at Rockport in August, 1960, shortly after young fish were first noted in July. Moreover, amazon fecundity at Lula Sams was much higher in 1961 than 1960. In 1960 the population was more dense until the ditch was dredged, after which it would take time for the environment to stabilize. Egg production was much greater in August and September, 1960, two months after the dredging. In 1961 reproductive senescence was not apparent until July after young first were noted in June.

The decrease in fecundity with size may also involve food supply. Basal metabolism needs should have priority over reproductive needs for the metabolites, because the female must live until the young are born or the metabolites used for reproduction are wasted. If food is in short supply the amount remaining from basal metabolism would be limited and fecundity reduced. The capacity to find and ingest food increases with size; however, this increase should be more arithmetic than the geometric increase in the basal metabolism needs as the female grows. Therefore, larger fish would not attain their full reproductive potential when smaller ones were still at full efficiency. The largest fish might have a basal metabolism that approaches the optimal ingestion potential and never have metabolites available for reproduction. Such a fish could become agonadal or reproductively senile. If environmental conditions were unfavorable, reproductive senility would develop in smaller females than under optimal conditions. Williams' (1957) discussion of senescence did not incorporate environmental factors that appear here to be extremely significant. Furthermore he proposed that "there should be little or no postreproductive period in the normal life cycle of any species," a happenstance that occurs in mollies and was demonstrated for *Gambusia* by Krumholz (1948).

It is likely that a shortage of food is a primary factor in preventing female amazons and sailfins from attaining their full genetic reproductive potential. If so, their use of the same primary food source would result in each species reducing the reproductive efficiency of the other. Comparison of reproductive senility at Central Avenue (Figs. 19 and 20) shows similar patterns

especially in August, 1960 and July, 1961, indicating that similar factors are involved. Although experimental data to confirm this hypothesis are lacking, I believe that food is often in short supply and that amazons and sailfins use similar foods so that each reduces the food available for the other.

As information on reproductive potential is essential to population dynamics, it is important to predict the number of eggs a given population produces per year. This is often done by counting the eggs in a sample of females and multiplying the results by the number of females of each size. This procedure may be safe if the samples are from the same breeding interval to which the extrapolated data are applied; however, it would be dangerous to apply it at other times. Calculations based on the reproductive potential of sailfin mollies collected at Rockport in July or August, 1960, would differ much more greatly than the normal statistical errors. Moreover, these data show that pooling collections for the comparative fertility studies would distort the data.

Summary

The amazon molly belongs to an unusual breeding system. Only one of the 33 phenotypic males appears to have been functional and all seem to be genetic females. Males of related species serve as "fathers" of the broods but the offspring show no sign of functional paternal chromatin. The possibly functional male amazon molly may have merely stimulated gynogenetic development. Under any circumstances the low male frequency in amazons and large fraction of nonfunctional males eliminates them as a major contributor to the production of offspring. Therefore, the breeding system includes two types of females and one type of male, all of which have the same number of chromosomes. The male and one of the female strains form a normal bisexual breeding system (the sailfin molly in the study area). The other female type (the amazon molly) contributes nothing to the bisexual system and uses the sailfin males as "fathers" of the next generation. Similar three-way breeding systems have been shown to occur in a few other organisms, but no previous study has been directed toward the way the system works in nature. The unisexual (amazon) females have a distinct reproductive advantage over the bisexual females, by producing few males. As the males are shared by the two types of females, any males produced would accordingly reduce the relative reproduction potential of that female's offspring. Some males must be produced however, or the entire system would stop. Unless a compensating relative increase in the reproductive potential of the bisexual females occurs, the bisexual would become extinct, and the unisexual species would soon follow. The necessity of sharing males provides a potential weak point in the unisexual life history. Natural selection on the

males would not result directly in modification of the unisexual's genome.

The unisexual and bisexual females have many similarities in their ecology. They live in shallow, quiet water and feed on periphyton. Areas with concentrations of aquatic vegetation contain large numbers of mollies, especially young. The sailfins tend to be more common in cooler water with emergent vegetation, but any habitat near Brownsville which includes one species is also occupied by the other. Sailfins are also known to occur in more saline waters than the amazons, but this habitat does not occur at the sample stations. Both species grow rapidly in warm months and slowly in cool months. The first broods in a year grow more rapidly than the later broods. Winter growth is nil with the possible exception of the smaller young of the year which compacts the size variation of that year class from fall to spring. Young fish grow much more rapidly than do older fish at the same season.

At Brownsville, amazon and sailfin females have similar reproductive potentials. Mature ova are first found in females of about 30 mm. standard length (two to three months old). Equal-sized females produce approximately the same number of eggs. Both species have a breeding season that extends from spring until fall, with earlier ovulation giving the amazons a slight reproductive advantage. The initiation of the breeding season appears to be in large part controlled by temperature. The number of ova produced by equal-sized females of the two species varies with the season. Relative fecundity increases in the spring to a mid-season peak and then decreases in the fall. Reproductive senescence and senility are most common in late summer and fall collections. These changes appear to correlate with light duration through available food. It is believed that each species inhibits the relative fecundity of the other since both use the same primary food supply.

Sailfin reproductive potential must obtain an advantage over that of the amazons in order to maintain the three-way breeding system. Females of the two species have no intrinsic differences that would favor the sailfins; in fact the only major difference (ovulation) favors the amazons. As the amazons "borrow" the sailfin males to initiate development of their young, this link is a logical weak point in their life history. A reproductive advantage using sexual interactions could be flexible and effectively keep the unusual breeding system in balance.

Courtship observations in the laboratory show a distinct preference for sailfin females over amazon females by sailfin males. This preference is greater in sympatric sailfin males than in those which were captured outside of the amazon's range. Therefore this preference has considerable selective value. This sexual discrimination should result in differential pregnancies favoring sailfins. An interspecific difference in the probability of more or less fecund females being pregnant is an additional reproductive advantage for sailfins. Pregnant sailfin females typically contain more eggs than non-

pregnant females in the same collection. Amazon females are the opposite in that the least fecund females are more likely to be pregnant. Part of the difference results from a decrease in probability of being pregnant with increasing length in amazon females. This decrease does not occur in sailfins and there is some evidence that size and pregnancy frequency are directly associated. The interspecific fecundity difference is also derived from the fact that, within a size group, pregnant sailfins have more eggs than non-pregnant ones. Among the amazons, however, the nonpregnant ones have the greatest number of eggs.

Male mollies are territorial. Probably the most fecund sailfins in a territory are courted first, then the less fecund sailfins, followed by the least fecund amazons and finally the more fecund amazons. This conclusion, based on studies of mature females, is supported by the great difference in the relative number of sailfin (more) and amazon (fewer) young collected early in the breeding season.

Under such circumstances a reduction in number of sailfin males would reduce the reproduction of amazons and have little effect on sailfin reproduction. The sex ratio of sailfins in the sympatric population at Brownsville favors females far more than at Rockport where amazons are absent, showing the strong selection for factors that favor sailfins. As a result, in Brownsville there is about one male for every four females. If the amazons are included, the sex ratio there would be between 1:10 and 1:100. Male frequency may limit the reproductive capacity of a population with such an unbalanced sex ratio. Partially pregnant mollies probably represent females that have not been inseminated recently so that sperm was available for only part of the egg complement. Partial pregnancy is most common in amazon females, the group which would be least frequently inseminated. Partial pregnancy is very rare at Rockport, where males are abundant and females should be inseminated frequently. Females inseminated when partially pregnant would produce multiple broods, or exhibit superfoetation. The occurrence of this phenomenon in amazons further indicates that only rarely are they inseminated.

The relative efficiency of amazon reproduction varies and is positively correlated with sailfin population density. Sailfin males that had inseminated all the female sailfins in their territory would then court and inseminate amazons. If amazons were scarce, most or all might be inseminated, and an increase in the abundance of amazons would result in a decreasing fraction being inseminated. This delicate balance could not be changed by the amazons as they are not involved in the inheritance of male preferences and if it were changed, the entire system would become extinct. Likewise, sailfins are somewhat bound to maintain the status quo. A decrease in male virility would have a serious effect on the amazons, but those males would leave

fewer sailfin progeny and the genes would be selected against. An increase in sexual preference could eventually free the sailfins from the amazons; however, males of every fish I know will attempt to court females of related species if homospecific females are unavailable.

APPENDIX I—CHROMOSOME NUMBER

by GEORGE E. DREWRY

I studied the chromosomes of *Mollienesia formosa* in an effort to test Meyer's (1938) assumption of diploidy in this form. Beatty (1957) demonstrated that a routine technique could be developed for counting chromosomes in the corneal epithelium of the mouse, and that this tissue exhibited a high degree of numerical constancy. The diploid number established in this way was in accordance with that of other tissues, including the haploid number from spermatogenic meiosis. Such a technique has obvious advantages over procedures based on the testis, being independent of the sex of the animal. Moreover, testicular tissues are scarce in functionally gynogenetic species. Swarup (1959) used a similar technique in his studies of triploidy in the stickleback *Gastersteus aculeatus*, but took his samples from the conjunctiva of the eye.

The corneal epithelium and the conjunctiva of *M. formosa* were studied, but all of the counts recorded were found in the thinner layers of the corneal epithelium. Mitotic activity within this tissue appears to be infrequent in the adult fish. Damage to the tissue seemed to stimulate cell division, and best results were obtained when the eyes were bathed momentarily in absolute ethyl alcohol ten to 18 hours before samples were taken.

Swarup (1959) used biopsy to obtain conjunctiva. In this study mollies were killed and the eyes carefully removed to a hypotonic solution for 20–40 minutes before fixing, a technique based on Venge's (1954) method for spreading the chromosomes of the rabbit blastocyst. The hypotonic solution used was Holfretter's solution with one-half the normal concentration of NaCl and no KCl.

At the end of this pre-treatment the epithelium was fixed by a dip into Carnoy's fixative (3 parts E + OH abs, 1 part glacial HAc) to facilitate its removal from the cornea. Small patches about 1 mm. square were placed in drops of stain-fixative (2% orcein in 1 part glacial HAc, 1 part lactic acid, 1 part H₂O) on microscope slides and covered with glass cover slips.

The spherical brass head on a steel insect pin was used to prepare the slide for inspection. The cells were separated by sharp taps above the piece of tissue, a technique which was not observed to disrupt cell contents. The tissue was squashed by working the fluid to the edge of the glass with light but firm strokes of the pinhead. If the quantity of fluid was correct the cells

were kept flattened by capillarity between the slide and cover glass. The chromosome spreading under optimum conditions was better than that obtained with a mechanical press (Beatty, 1957) and only a few cells were clearly disrupted during squashing. The technique failed to work with stain-fixatives which did not contain lactic acid.

The testes of two male *Mollienesia latipinna* (Austin, Texas stock) and one male *M. mexicana* (Huasteca Canyon, near Monterrey, Mexico) were studied along with corneal epithelium.

The testes were removed at the same time as the eyes, cut into small cubes and given the same hypotonic pre-treatment. They were then killed and fixed by soaking from 3 to 24 hours in the staining solution. (Fixatives containing alcohol were found to toughen the tissue and prevent suitable squashing). The mounting and squash techniques were otherwise the same as for corneal epithelium.

The results of 14 mitotic counts on two female *M. formosa* reared in the laboratory from Brownsville stocks, 8 meiotic counts on the male *M. mexicana* and 9 meiotic and 2 mitotic counts on the two male *M. latipinna* are given in Table IX. The estimated accuracy of count is based on criteria given by Beatty (1957).

The actual diploid chromosome number of the amazon molly is not established by these counts. The excellence of the single count shown in Fig. 23A has led me to believe that the number is $2n=46$ consisting exclusively of telocentric rods.

Rearrangement of chromosomes according to size (Fig. 23B) illustrates my doubt that the chromosomes are dissimilar enough for detailed cytomorphological identification. The disparity in size between the two largest chromosomes may be an artifact of squashing but was noted in several counts and could indicate heterozygosity if real. This heterozygosity could indicate a functional allodiploid condition or the presence of nonfunctional paternal chromatin that differs slightly in certain morphologic features from the functional maternal chromatin. The apparent difference is not in accord with a hypothesis of fusion of identical reduced nuclei.

The work of Wickbom (1943) on *Molliensia velifera* and *M. sphenops*, and my work on *M. latipinna* and *M. mexicana* indicate that the most common diploid chromosome numbers in the genus are between 40 and 50. Thus it is reasonable to assume that *M. formosa* is diploid. The actual number of chromosomes indicated for *M. latipinna* is $2n=46$, $n=23$, the same number Wickbom reported for *M. velifera* and *M. sphenops*. Although my counts for *M. mexicana* are inconclusive, this species may have $2n=46$ chromosomes. Meyer (1938) reported $2n=36$ for *M. sphenops* and *M. latipinna*. Wickbom has discussed the discrepancy in *M. sphenops*. As the same discrepancy holds for *M. latipinna*, I agree with Wickbom's reservations as to Meyer's count.

TABLE IX
Chromosome Counts of Three Species of Mollienesia

Estimated Maximum Accuracy of Count	Estimated Haploid Number of Chromosomes							Estimated Diploid Number of Chromosomes				Total Sample
<i>M. mexicana</i> 1 male												
	19	20	21	22	23	24	25					
exact	1°			1								
± 1		1°		1	1							
± 2						1 ^a						
± 3						1 ^a	1 ^a					
Total	1	1		2	1	2	1	8				
<i>M. latipinna</i> 2 males												
		22	23					44	45			
exact			3									
± 1		1	3						1			
± 2			1									
± 3		1						1				
Total		2	7					1	1	11		
<i>M. formosa</i> 2 females												
						38	44	45	46			
exact						1°			2			
± 1								3	4			
± 2							2		1			
± 3								1				
Total						1	2	4	7	14		

* Cells probably disrupted
^a Tetrads starting to separate

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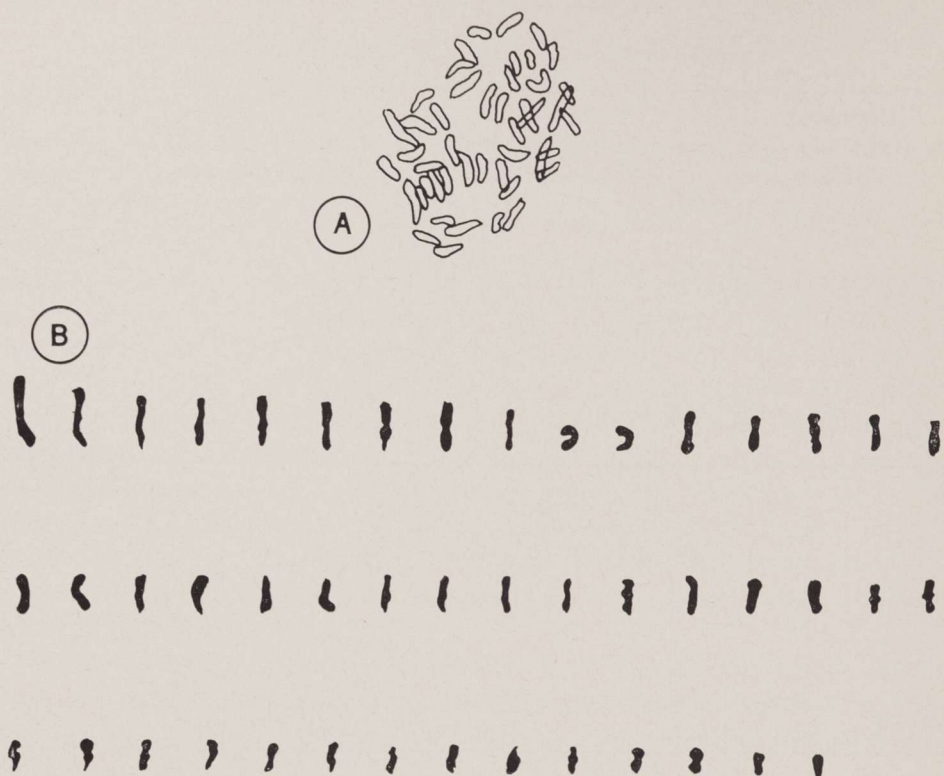


FIG. 23. Chromosomes from the corneal epithelium and an amazon molly from Brownsville stocks. A. Camera lucida drawing. B. Arrangement of chromosomes by size, showing the gross morphology.

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